HEMATOLOGICAL AND SERUM CHEMICAL CONSTITUENTS IN PANTROPICAL SPOTTED DOLPHINS (STENELLA ATTENUATA) FOLLOWING CHASE AND ENCIRCLEMENT

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ABSTRACT

Analysis of blood samples produced a comprehensive dataset on hematological and serum and plasma chemical constituents in spotted dolphins from the ETP. Several of the analytes, notably the catecholamines and ACTH, have not previously been investigated in this species, and therefore represent new and important baseline information, particularly in relation to the adrenal-mediated stress response. Apparently significant elevations in the circulating levels of these constituents help to define the degree of stress experienced by the dolphins after chase and encirclement by a tuna-fishing vessel, although correlation between the severity of the stressor and deviation of blood results are tenuous, and few true baseline data for unstressed spotted dolphins are available from which to gauge how much these values deviate from resting levels. Still, the persistence of high levels of ACTH suggests that the HPA axis continues to be stimulated while the dolphins are confined in the net. Dolphins also exhibited moderately elevated levels of enzymes indicative of muscle damage following the exertion of the chase, and some showed deviations in constituents suggesting metabolic acidosis.

Efforts were made to obtain serial samples from individuals repeatedly captured, and to assess recovery from the acute stress response by examining specific diagnostic indicators in blood. Logistic impediments in the field resulted in only 10 recaptures, but only two of these dolphins were blood-sampled when first handled. This significantly limits the strength of any conclusions that can be reached regarding the animals' ability to cope physiologically with this perturbation. Certain changes were noted signaling a stress response, but none that suggested distress at the time of second capture.

INTRODUCTION

The analysis of formed elements and chemical constituents in blood has long been a mainstay of veterinary diagnostics, and has been especially useful for species such as cetaceans, for which other approaches have either lagged or are impractical to apply (Bossart *et al*. 2001). Changes associated with stress and disease can be recognized against robust baseline data that have been published for many species of small cetaceans (Ridgway *et al*. 1970, MacNeill 1975, Cornell 1983, Cornell *et al*. 1988, Asper *et al*. 1990, Koopman *et al*. 1995, 1999, St. Aubin *et al*. 1996, 2001, Bossart *et al*. 2001). However, baseline blood data for free-ranging, live-captured spotted dolphins, *Stenella attenuata*, in the Eastern Tropical Pacific Ocean are meager, and consist primarily of serum chemical analysis of 44 samples collected in 1977-78 and 4 samples in 1993; there are no corresponding hematological data. The current study therefore had three objectives: expand the baseline dataset in terms of both the number of specimens and the variety of analytes, examine these data for evidence of acute responses to the stress of chase and encirclement, and evaluate findings in repeatedly captured dolphins for evidence of additive changes that would signal an inability to recover from the stress represented by each capture.

A substantial literature exists on blood constituents as indicators of the stress response in cetaceans (Orlov *et al*. 1988, Thomson and Geraci 1986, St. Aubin and Geraci 1988, 1989, 1992, Ortiz and Worthy 2000). Following the general relationships in other mammals, the stress response in marine mammals can be tracked by monitoring the activity of selected endocrine glands, and the effects of their secretions on other aspects of

homeostasis (Curry 1999, St. Aubin and Dierauf 2001). The sequence of events follows a broadly recognized time course in response to a nominal stressor, based on the organism's need for immediate or longer term adjustments to meet the demands imposed by a particular challenge. These changes are for the most part adaptive and beneficial, and are in fact necessary for organisms to survive. However, exaggerated or repeated perturbations leading to distress or a chronic state of stress are not adaptive, and can compromise health, reproduction, and ultimately survival.

Studies on the stress response in wildlife are challenging because the activities necessary to collect specimens such as blood generally represent a stressor capable of perturbing certain indices. Most hematological and serum chemical studies on wildlife are based on single samples representing a "snapshot" of the individual's condition (Beltran *et al*., 1991; Dunbar *et al*., 1997; Vogel *et al*. 1999; Borjesson *et al*. 2000), with the perturbations inherent in sample collection viewed as an unavoidable but sometimes controllable variable. Still, samples obtained in this manner have limited prognostic value, since it is important to know if any specific abnormal result in an individual is trending toward resolution or represents a worsening condition. In some instances, it is difficult to recognize an abnormal value against a population mean that is derived from a heterogeneous sample representing all ages, both sexes, and individuals in differing states of health. For this reason, health monitoring in captive specimens generally references normal values established for each individual. The ability to use blood values as indicators of acute and chronic stress in the present study therefore depended heavily on the repeated capture and sampling of individuals to properly assess trends in relation to an individual's "baseline" values. Statistical comparisons with the population data would likely miss important changes in resampled dolphins.

Blood analyses featured prominently in addressing a central question of the Chase Encirclement Stress Studies (CHESS) – does repeated chase and encirclement of spotted dolphins result in stress-related tissue damage and functional impairment? An expert panel convened at the Southwest Fisheries Science Center in January, 2001, recommended a set of constituents for analysis to provide the most useful information on the physiological status of dolphins repeatedly pursued and captured during the course of tuna fishing operations. The suite of analyses included routine hematological and serum chemical constituents, and hormones specifically targeting adrenal (both medulla and cortex), thyroid, gonadal, and pituitary functions. It was recognized that the number of analytes that could potentially be examined was much larger, but many of these were excluded because the methodology had yet to be developed and validated for the species in question (e.g., peptide hormones such as prolactin) or the virtual absence of comparative data precluded meaningful interpretation (e.g., troponin). Ultimately, conclusions derived from the data collected for spotted dolphins would rely heavily on extrapolations from clinical situations for other species of small odontocetes.

An independent scientific peer review of this work was administered by the Center for Independent Experts located at the University of Miami. Responses to reviewer's comments can be found in the Appendix.

METHODS

Blood collection techniques

Dolphins freely swimming within the seine net were grasped by swimmers and maneuvered into inflated rubber rafts moored to the cork line of the net. Blood samples were drawn with the dolphin restrained in the raft, generally within 5 minutes after they were captured by the swimmers. Samples were collected variably from the tail flukes or the ventral caudal peduncle using 19 or 21 gauge needles (intravenous butterflies, or hypodermic needles attached to extension tubes). Blood was collected directly into Vacutainer® tubes (Becton Dickinson, Franklin Lakes NJ) treated with either Na-heparin or EDTA, or untreated tubes containing separation gel for harvesting serum. Samples were placed immediately on cold packs and transferred to the air conditioned shipboard laboratory within 1 hour.

In the laboratory, samples were held on ice and processed uniformly in the same sequence after each set. First, heparinized blood was centrifuged for 5 min at 3500 rpm (1500 g) using a Triac 0200 centrifuge (Clay Adams Division, Becton Dickinson and Co., Parsippany, N.J.), to harvest plasma for catecholamine analysis. Plasma samples were frozen at –80°C, typically within 30 min after returning to the lab. Next, blood treated with EDTA was centrifuged as for the heparinized samples, and plasma harvested and frozen for ACTH analysis within the following 30 min. Finally, clotted samples were centrifuged and sera aliquotted and frozen during the ensuing 2 hours. Aliquots of all specimens were stored in 2 mL cryovials uniquely numbered so as not to reveal the identity of the subject or the date of collection to the intended laboratories.

Concurrent with the processing of plasma and serum, EDTA-treated blood was analyzed for hematological constituents using an ABCDiff® semi-automated analyzer (Heska Corp., Fort Collins CO). Each run began with a control determination (Monitrol, ABX Diagnostics, Irvine CA) to establish the performance of the instrument. All data from dolphin samples were generated after obtaining acceptable results from a control sample. Specimens were placed on a tube rocker to ensure mixing prior to analysis. All determinations were performed in duplicate, and accepted only if results for white blood cell count (WBC), red blood cell count (RBC), hemoglobin (Hb), hematocrit (Hct) and platelet count (Plt) showed no more than 5% difference between a pair of runs. Rarely, a third determination was needed to resolve a questionable result, which was usually a case of operator error, and the obviously erroneous data were ignored. In addition to the aforementioned constituents, the analyzer also produced results for mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean platelet volume (MPV) and red cell distribution width (RDW). Results are reported as the mean of the duplicate determinations.

Blood smears were prepared in triplicate and stored for later evaluation at the Diagnostic Laboratory of the College of Veterinary Medicine at Cornell University, Ithaca, NY. All slides were labeled only with a unique number that did not reveal the animal identity or the date of collection. Differential counts were based on examination of 200 cells on one slide per blood draw. Duplicate smears from 6 randomly selected draws were submitted blind to the laboratory to evaluate the reproducibility of the estimates. Four of the six duplicates returned nearly identical results, while the other two pairs showed differences of up to 15% in the most numerous cell classes. Recounts of those slides produced acceptable replicates, with mean values closely matching the means derived from the original counts.

Microhematocrits were determined in duplicate using standard techniques as a means of verifying the performance of the automated hematology analyzer. Paired microhematocrit tubes yielded either identical measures or differed by only one percent (e.g., 46% and 47% as duplicate hematocrits). Results showed a highly significant correlation to those reported by the analyzer ($r^2 = 0.76$, $p<0.001$). Fibrinogen was determined in duplicate using the heat precipitation method of Millar *et al*. (1971). Results are reported as the mean of duplicates, which showed an average difference of 5.8 ± 5.5% (median – 4.8%, range – 0-27.1%, n=48).

Comparative Data

Due to the relative absence of published data for certain critical stress-associated constituents in blood from any cetacean species, samples were obtained for analysis of catecholamines, ACTH and cortisol from free-ranging bottlenose dolphins, *Tursiops truncatus*, captured in June, 2001, as part of a long-term research project directed by R.S. Wells in Sarasota Bay, FL. Blood collection and handling procedures were virtually identical to those later employed in the Eastern Tropical Pacific, and the samples were analyzed at the same laboratories for the respective constituents. The principal differences between the two sets of data were that the *Tursiops* experienced little or no chase prior to encirclement, and virtually all the samples were collected within 60 minutes after deployment of the net.

Chemistry

Serum samples were analyzed for 28 constituents (electrolytes, metabolites, enzymes) and 4 derived indices (A:G, Na:K, % saturation, anion gap) at the Diagnostic Laboratory of the College of Veterinary Medicine at Cornell University, Ithaca NY, using an Hitachi 917 multichannel auto analyzer (Roche Diagnostics Corp., Indianapolis IN). The samples were run together as a batch, and were ultra-centrifuged prior to analysis to clear the samples of chylomicra and residual fibrin. Normal and abnormal controls, run each day as a standard operating procedure for the laboratory, yielded results within the expected ranges for all constituents. Six aliquots of a pooled sample of *Stenella* serum were submitted blind to the laboratory and interspersed within the batch of samples to establish the confidence in the results of single determinations on the test samples. Coefficients of variation (cv) for the 32 determinations had a median value of 1.35%, with three constituents showing cv's in the range of 5-10% (bicarbonate, creatinine and uric acid) and one (anion gap) at 11.36% as the highest value for this parameter.

Thirty of the serum samples were submitted to the Diagnostic Laboratory at Cornell University for protein electrophoresis to quantify protein fractions. Specifically, ten specimens from recaptured dolphins were compared to 20 specimens from presumed first-captures, with the latter group selected to represent individuals with low and high globulin levels.

Hormones

Hormones were analyzed using commercially available kits from Diagnostic Products Corp. (DPC, Los Angeles CA), Nichols Institute Diagnostics (San Juan Capistrano CA) or Polymedco (Cortlandt Manor NY), following the manufacturer's procedures. Small variations, such as modifying the amount of sample, were sometimes introduced to improve

the performance of the kits, and are noted below. As for the serum chemistry analyses, six aliquots of a pooled sample of *Stenella* serum were submitted blind to the laboratory and interspersed within each batch of samples to establish coefficients of variation for the various hormonal determinations (listed below).

Hormone	Method	Supplier	Pooled Sample cv	Comments
Adrenocorticotropic Hormone (ACTH)	Chemiluminescent enzyme immunoassay (Immulite®)	DPC	2.0%	Manufacturer's protocol
Aldosterone	Solid phase ^{125}I radioimmunoassay (Coat-a-count®)	DPC	4.9%	Manufacturer's protocol
Cortisol	Chemiluminescent enzyme immunoassay (Immulite®)	DPC	9.9%	Manufacturer's protocol
Total Thyroxin (T4)	Solid phase ^{125}I radioimmunoassay (Coat-a-count®)	DPC	7.9%	Sample volume doubled; 10 min incubation after addition to Ab tube; additional standard of 0.5 μg/dL
Total Triiodothyronine (T3)	Solid phase ^{125}I radioimmunoassay (Coat-a-count®)	DPC	17.4%	Manufacturer's protocol
Hormone	Method	Supplier	Pooled Sample cv	Comments
Free Thyroxin (fT4)	Equilibrium dialysis Solid phase ^{125}I radioimmunoassay	Nichols Institute Diagnostics	8.4%	Manufacturer's protocol
Reverse Triiodothyronine (rT3)	Double antibody ^{125}I radioimmunoassay	Polymedco (Serono)	17.7%	Manufacturer's protocol
Testosterone	Solid phase ^{125}I radioimmunoassay (Coat-a-count®)	DPC	7.7%	Manufacturer's protocol
Progesterone	Solid phase ^{125}I radioimmunoassay (Coat-a-count®)	DPC	4.8%	Manufacturer's protocol
Estradiol	Solid phase ^{125}I radioimmunoassay (Coat-a-count®)	DPC	13.6%	Ethel ether extraction; ^{3}H-estradiol used to test extraction efficiency and determine correction factor

Epinephrine (E), norepinephrine (NE), and dopamine (DA) were determined at ARUP Laboratories (500 Chipeta Way, Salt Lake City UT) using High Performance Liquid Chromatography with Electrochemical Detection (HPLC-EC). Samples of heparinized plasma (2 mL) were extracted with 30 mg of alumina (Biorad) in a conical centrifuge tube containing 200 μL of working internal standard and 1.0 mL TRIS buffer. Tubes were vigorously shaken then centrifuged at 2500 rpm for 5 min. After removing the supernatant, the alumina was twice washed with 1.0 mL of HPLC grade water before adding 150 μL of 0.1M phosphoric acid. The solution was vortexed for 30 sec to extract the catecholamines from the alumina, and then centrifuged at 2500 rpm for 2 min. Fifty μL of the supernatant was injected onto a Plasma Catecholamine Analytical Column (Biorad). Quantitation of E, NE, and DA is based on comparing peak height ratios relative to an internal standard in both the unknown sample and a plasma calibrator specimen. Ten replicate samples were submitted blind to the laboratory to establish the precision of the assay. Average difference from the mean value for the replicates was 6.9 ± 6.5% (range 2.6-19.2%) for NE, 10.4 ± 10.9% (range 1.4-28.6%) for E, and 8.9 ± 7.8% (range 0.4-26.2%) for DA.

Statistical Analysis

Statistical analysis was performed using Statistix7 (Analytical Software, Tallahassee, FL), and include ANOVA, ANCOVA, Spearman Rank correlations and Pearson correlations, as appropriate. Baseline data were examined for outlying values, for the effects of age and sex, and for the influence of time between specific events and collection of blood. Time intervals were established relative to the follows events:

Event 1 – Start chase (generally marked by the appearance of helicopter above the herd)
Event 2 – Speedboats in (intensification of the chase)
Event 3 – Let go (net in the water)
Event 4 – Rings up (seine fully pursed)
Event 5 – Tie down (most of the net recovered on board and secured. The time at which normal back down procedures are begun)

Ultimately, the statistical evaluations based on the time of blood sampling relative to the start of the chase (Interval 1) were considered the most useful in terms of studying the chronology of the acute stress response in *Stenella* since it offered the broadest range of times, spanning 2.5 hours, and was related to the earliest recognized stressful stimulus to the dolphins. An important limitation of this approach is that the shortest time between the start of the chase and collection of a blood sample was over 1.5 hrs, enough time for some of the responses to have occurred and normalized if subsequent events elicited no further response.

RESULTS

Baseline Data

The majority of the blood samples were collected from relatively calm dolphins manually restrained in the rafts. Occasionally, the animals offered some resistance, contributing to insufficient draws to support all of the intended analyses. In all, blood for at

least some analyses was obtained from 61 different dolphins, 53 of which were presumed to have been captured for the first time during the course of this study. It is likely, however, that some of the latter individuals had recently been chased and encircled, potentially influencing the blood constituents examined. However those animals cannot be identified with certainty and are therefore could not be removed from the baseline dataset for the population (Tables 1-3).

The serum chemistry data were compared with those collected in 1977-78, with 15 of the 23 constituents and calculated ratios showing significant differences between the two datasets (Table 2). Modifications in analytical methodology likely account for the discrepancies, particularly for the enzymes ALT, AST, and CK. Higher K concentrations in the current dataset translated to a lower Na:K ratio, and were probably correlated with higher Cl in these dolphins as well. Higher globulins measured in 2001 are also reflected in higher total proteins for that group. Both Ca and P were significantly higher in the 2001 samples, as were cholesterol and triglycerides. The latter is highly influenced by recent feeding (see below), a variable that could not be controlled in the selection of dolphins to be sampled. Calcium levels vary with age of the animal (see below), which also was not matched in the respective samples. Differences in creatinine and total bilirubin were so small as to be clinically meaningless. The four samples obtained in 1993 produced values for all constituents (Reinhardt, unpublished data) that were within the ranges reported for the current study.

Data for each constituent were examined for outliers to identify individuals exhibiting unusual profiles. In addition, values in the first and last 10^{th} percentiles were recognized as potentially signaling an especially perturbed state, to be considered in the overall assessment of an individual's physiological status. Twenty-six of the dolphins sampled on nominal first capture showed outlying values in one or more of the blood constituents analyzed. Of these, 18 had only one outlying result, while the remainder showed up to eight. In all, data for 28 of the blood constituents included values determined to fall outside of the expected distribution (Table 4). Particular attention was given to associations among complementary indices (e.g., enzymes associated with muscle damage, electrolyte profiles). Scatter plots of these data highlighted how some individuals were clearly distinguished from the remainder of the chased and captured population (Figs. 1-3)

Correlations among Constituents

Significant correlations were noted among many of the constituents. Some of the measures are understandably related (e.g., RBC counts with hematocrit and hemoglobin, iron with percent saturation of iron binding proteins) since one is for the most part determined by the other. Others reveal some dynamic interactions, particularly for the hormones. Among the thyroid hormones, there were highly significant ($p<0.001$) correlations between T4 and T3 ($r = 0.643$), T4 and rT3 ($r = 0.560$), and fT4 and each of T4 ($r = 0.829$), T3 ($r = 0.628$) and rT3 ($r = 0.414$, $p<0.01$). Norepinephrine and dopamine concentrations were significantly correlated ($r = 0.585$, $p<0.001$), but neither was associated with levels of epinephrine (Fig. 3). Aldosterone levels were positively correlated with those of ACTH ($r = 0.379$, $p<0.01$) and cortisol ($r = 0.410$, $p<0.01$), but cortisol showed no significant relationship to ACTH. Total globulin was significantly correlated with the gamma ($r=0.9499$, $p<0.001$), beta-2 ($r=0.7412$, $p<0.001$) and alpha-2 ($r=0.5577$, $p<0.05$) fractions.

Spearman Rank correlations were examined among values for electrolytes, bicarbonate and anion gap. Significant negative correlations ($p<0.001$) were found between bicarbonate and each of sodium, chloride, and anion gap, but not with potassium. Sodium was positively correlated ($p<0.01$) with both chloride and anion gap. Among the serum enzymes, AP was positively correlated with AST and CK; AST was also positively correlated with LDH. No significant relationships were noted between enzyme levels and indices of acid-base balance.

Acute Changes in Blood Constituents - Effect of Sampling Time

The unpredictability of the chase and logistics of net deployment and retrieval were variables did not allow for standardization of the time of blood collection, which was achieved at the earliest opportunity. Considerable overlap existed from set to set in the time represented by these event markers (Table 5, Figure 4), contributing to the variability in those constituents known to show temporal adjustments following stressful stimuli. Changes in constituent concentrations were expected as a function of time after exposure to the stress of chase and encirclement, following a chronology specific to each particular measure. Given the marked difference in the amount of time that elapsed between the different activities associated with purse seining (boats in the water, net closure, etc.) and the collection of blood samples, the baseline data derived from those samples were examined for temporal effects.

Pearson correlations within each interval showed significant ($p<0.05$) temporal correlations for some of the hematological determinations, as follows:

Interval 1: (onset of chase) lymphocytes, MCHC, platelets, WBC
Interval 2: (speedboats in) lymphocytes, eosinophils, platelets, WBC
Interval 3: (let go) lymphocytes, eosinophils, MCH, platelets, WBC
Interval 4: (rings up) eosinophils, platelets, WBC
Interval 5: (tie down) lymphocytes, eosinophils, WBC

All correlations were positive with time, except for MCH. Significant correlations with time were also evident for 10 of 33 serum chemical constituents and 3 of 10 thyroid, adrenal and pituitary hormones, as follows:

Interval 1: Ca, Cl, glucose, Na:K, K, Na, urea, uric acid, T3, fT4,
Interval 2: Ca, Mg
Interval 3: Ca, Cl, Na, fT4
Interval 4: Ca, total bilirubin, glucose, Na
Interval 5: Na, Cl

Correlations were positive for all except total bilirubin, Cl, Na:K, and Mg. Only total bilirubin and Mg were not among the constituents showing significant correlations with time after the onset of chase (Interval 1), but appeared when other events were used to mark the time interval. Time after the onset of chase was also examined for its effect on the relationship between ACTH and both cortisol and aldosterone, but no significant temporal trend was determined in the ratio of ACTH to either steroid (Figs. 5 and 6). There was no significant correlation with sampling time for any of the catecholamines (Figs. 7 and 8).

Gender and Age Effects

Gender and relative age influenced a number of hematological and serum chemical measures, both individually and through interaction (Tables 6 and 7). A combination of body length, serum hormones and coloration pattern were used to distinguish mature from immature dolphins. Still, the distinctions between mature and immature animals were sometimes difficult to make, and so the values may not be absolute for each category. A minimum length criterion of 180 cm was used for females to establish a greater than 50% probability of being sexually mature. Accordingly, 19 of the 25 females sampled on first capture were mature. Of the males, 18 of 26 were considered mature on the basis of meeting at least one of three criteria: fused coloration pattern, body length > 190 cm, or testosterone> 5 ng/mL.

For the most part, the differences in the results of blood analyses were small and without clinical significance. Higher levels of ALP activity, calcium and thyroid hormones in younger animals were expected. Lymphocyte counts, as well as their relative proportion among leucocytes, along with cholesterol, CK, TIBC and UIBC were also higher in immature dolphins. Gender-related differences were less consistent, with only creatinine, rT3, and WBC showing uniformly higher mean levels in males compared with females of both age classes; MCH was higher in females.

Time of Day

A number of the serum samples, particularly those collected during the morning, were visibly lipemic, suggesting recent feeding. Blood samples drawn between 0930 and 1130 (n=31) had significantly higher levels of urea (74.1 ± 8.7 vs. 58.8 ± 10.9 mg/dL, $p<0.001$) and triglycerides (230 ± 127 vs. 152 ± 134 mg/dL, $p<0.05$) than those collected between 1300 and 1700 (n=22) (Fig. 9). Cholesterol concentrations showed no significant diel pattern.

Effect of recapture

Ten dolphins fitted with either a roto- (9) or "bullet" (1) tag were blood sampled at the time of known second capture; six on the day following application of the tag, one after two days, and three after three days (Tables 8-10, Appendices 1 and 2). Animals fitted with various transmitter and data-logging tags for tracking or for studies on dive behavior, swim speed, or heat flux were not considered in this analysis because these larger instruments represented some additional stress not normally associated with tuna fishing operations, confounding the interpretation of hematological and serum chemical changes attributable with simple chase and encirclement. Only two of the ten recaptured dolphins with roto- or bullet tags were blood sampled when first caught, significantly limiting the availability of individual reference data against which to measure changes in blood constituents.

The pattern of findings in the two resampled dolphins showed few similarities (Table 7). One (D67 - bullet tag, sampled after 1 day) showed decreased Cl, P, Fe, % saturation of IBC, UIBC, glucose, urea, fibrinogen, cholesterol, triglycerides, LDH, aldosterone, T3, T4, fT4 and testosterone (Table 8, Appendices 1 and 2). Increased UIBC, creatinine, total and direct bilirubin, and rT3 were also noted. Changes in the roto-tagged dolphin (D34) sampled after 2 days were observed in many of the same constituents, but often in the opposite direction. Increases, rather than decreases, were evident in P, Fe, %

saturation of IBC, cholesterol, triglycerides, cortisol and T3, while UIBC and cortisol fell rather than rose. Levels of urea, fibrinogen, LDH, aldosterone, T4, fT4, rT3, creatinine and bilirubin, which were all noticeably altered in D67, were essentially the same on recapture in D34. Similar decreases occurred in Cl and glucose occurred in both animals. The decline in progesterone and estradiols in D34 mirrored that of testosterone in D67.

Both of the recaptured and resampled dolphins showed modest changes in some white cell counts, but with little effect on the total number of white blood cells. The bullet-tagged dolphin sampled after 1 day (D67) exhibited neutrophilia that was offset by eosinopenia, while the roto-tagged dolphin sampled on the second day (D34) showed a neutropenia that was balanced by lymphocytosis (Appendix 1). The leucogram for D67 was also distinguished by the presence of band cells. No changes were noted in erythrocytic indices in either dolphin.

Eight dolphins were tagged on first capture while restrained in the water alongside an inflatable raft, and only blood sampled at the time of the second capture. Hematological and serum chemical data for these individuals are reported in Appendices 1 and 2, and combined with data from D67 and D34 for comparison of recaptured dolphins with the baseline population (Tables 8 and 9). For the purposes of this analysis, data from D67 and D34 were excluded from the first capture statistics to maintain the discreteness of the two groups. The age and maturity composition of the recaptured group (3 mature females, 5 mature males, 2 immature males) did not differ substantially from that of the first capture sample to which it was compared (19 mature females, 6 immature females, 17 mature males, 8 immature males), other than the absence of immature females and a slight bias toward males.

ANOVA comparisons between the 10 dolphins and the baseline data without consideration of the time interval between captures revealed significantly lower CK, globulins, T4, T3, Fe, and % saturation of IBC, and higher A:G and rT3:T3 ratios in the recaptured animals (Table 8). The differences in globulin concentrations were determined by electrophoresis to occur primarily in the gamma globulin fraction.

Six of the recaptures occurred after 1 day, allowing a tenuous comparison with the larger population based on the same time interval between captures. Most of the statistical differences noted in the broader comparison were lost, and the differences were limited to CK, A:G and T4 (Table 9). None of the differences can be explained by age and gender biases in the sample. In fact, the slight over-representation of males in the recapture samples should have influenced the values in a direction opposite to the observed trends. The significant differences were maintained when comparisons were made between the recaptured group and a subset of the first-capture sample randomly selected and matched according to age, sex, and sampling time interval after capture. There were no statistical differences in any hematologic parameter, whether only six or all ten recaptured dolphins were considered.

Six dolphins were fitted with data-logging telemetry tags for concurrent studies and to allow recapture of groups that included previously sampled but less-invasively tagged animals. Blood samples taken from these animals on recapture were not included in the present analysis because the effect of the tag was considered to be a stressor that bore little connection to the activities of the tuna fishery. This was confirmed by the finding of indications of an acute inflammatory response in many of these individuals. Coincidentally, however, three of these individuals were among those with the most

outlying results on first capture. The most notable of these animals (D42), which had 8 outlying determinations, showed unremarkable values for anion gap, bicarbonate, sodium, epinephrine and norepinephrine when recaptured.

DISCUSSION

The analysis of blood samples collected from spotted dolphins in the ETP has significantly augmented the data available for routine hematological and serum chemical constituents for this species, and contributed new information for hormones such as ACTH and the catecholamines. From this, we can gain some insights into the short-term physiological responses to the stress of chase and encirclement as practiced by members of the tuna fishing industry. However, logistics and the lack of group cohesion in *Stenella attenuata* interfered with our ability to repeatedly resample individual dolphins and thereby obtain sufficient information to address the question of recovery and chronic effects. It was recognized at the outset of the study that it would be difficult to identify the cumulative effects of repeated capture during a short time period by statistical comparisons between the recaptured animals and some baseline for the population. Individual variation, whether due to factors such as age, gender, reproductive status, or pre-existing health disorders results in typically wide ranges for any number of constituents, obscuring meaningful changes in a particular animal. As a result, this study can only identify characteristics of the stress response of spotted dolphins and compare them to our understanding of these processes in small odontocetes and other mammals.

The collection of "baseline" hematological and serum chemical data, here defined as the values determined in dolphins presumed to have been captured for the first time in an undetermined period of at least several days to perhaps some weeks, presented certain challenges that both complicate the data and represent opportunities for useful analysis. A major confounding variable in obtaining such data from any wild animal is the approach used to capture and restrain the individual. Exertion and stress produce well-recognized changes in blood constituents, and these changes develop over time scales specific to each constituent. The ideal sample is one obtained as soon as possible after the initial perturbation, with minimal physical or psychological trauma to the individual. The logistics involved in collecting samples in the present study were such that it was impossible to collect blood from dolphins any sooner than 100 minutes after the disturbance caused by helicopter overflight. In some instances, this time interval was over 4 hours long. The dataset therefore includes values that deviate significantly from what would be considered as a true baseline or resting level. This limitation was also recognized when the study was designed, but accepted as an inherent element common to each successive capture of tagged individuals. Cumulative changes reflecting a transition to a state of chronic stress would therefore have to be evident above and beyond those due to the immediate response to chase and encirclement.

The data represented here as baseline for spotted dolphins provide indications of physiological adjustments consistent with an acute stress response, though the intensity of this response can only be evaluated relative to resting levels for other species of odontocetes. The primary indicators of such a response in odontocetes and other mammals are adrenal hormones (both glucocorticoids and catecholamines), glucose, leucocyte counts, iron and thyroid hormones. Changes would be expressed to a greater or lesser degree

within the time window during which our samples were collected, and in fact were expected to show some variation during the course of that period. The most obvious indications of an acute stress response in the spotted dolphins are found in the levels of cortisol and glucose. Cortisol values (5.06 ± 1.25 μg/dL , range 2.57 – 7.95 μg/dL) were higher than for any other reported baseline values for odontocetes (St. Aubin and Dierauf 2001), with the exception of harbor porpoises, *Phocoena phocoena*, sampled after periods of 1-3 days of entrapment in fishing weirs and then captured by seine net over a period of up to 2 hours (Koopman *et al*. 1995). The prolonged stress experienced by the harbor porpoises resulted in mean values of 8.8 ± 3.3 μg/dL (range - 4.2-19.9 μg/dL), whereas a typical resting value for odontocetes is less than 4 μg/dL. Free-ranging bottlenose dolphins captured by net encirclement and sampled within 90 minutes had levels of 2.6 ± 0.8 μg/dL, with a range of 1.2 - 4.1 μg/dL (St. Aubin *et al*. 1996), 2.4 ± 1.13 μg/dL, range 0.8 – 5.44 (Wells and Reinhardt, unpublished), and 2.8 ± 1.0 μg/dL, range 1.0 – 5.6 μg/dL (Ortiz and Worthy 2000). The later sampling time relative to the initiation of the stress response in this study likely accounts for the intermediate levels of cortisol that were observed. Stimulation with exogenous ACTH produces elevations in cortisol that peak after 1-2 hours in bottlenose dolphins and beluga whales (Thomson and Geraci 1986, St. Aubin and Geraci 1990), which corresponds to the average time between let go and blood sampling in this study.

Hyperglycemia is a generally accepted consequence of elevated cortisol levels, and results from suppression of insulin and stimulation of gluconeogenesis in the liver (Orth and Kovaks 1998). The value to the organism under challenging conditions is to increase the availability of this substrate to vital tissues such as the brain. Glucose levels in the spotted dolphins (136 ± 24 mg/dL, range - 92-215 mg/dL) were elevated above those reported for most odontocetes, which typically average approximately 110 mg/dL (Bossart *et al*. 2001). As further evidence of stress-associated hyperglycemia in odontocetes, levels in weir-impounded harbor porpoises averaged nearly 200 mg/dL (Koopman *et al*. 1995). Belugas captured and taken into captivity showed an increase from approximately 95 to 150 mg/dL during the first 3-5 hours (St. Aubin and Geraci 1989). The time frame during which the samples were collected from the spotted dolphins coincides with the documented onset of cortisol-induced hyperglycemia in other species.

Cortisol secretion by the adrenal cortex is controlled by circulating levels of ACTH produced by the adenohypophysis, or anterior pituitary. There are no published data on the latter hormone in the blood of any cetacean, however earlier work on mysticete pituitary extracts determined that the hormone in fin whales, *Balaenoptera physalus*, is identical to that in humans (Kawauchi *et al*. 1978). On this basis, a commercially available assay for the hormone was used for the first time to determine ACTH levels in this study, and also applied to specimens from bottlenose dolphins and beluga whales for purpose of comparison. The performance of the assay was judged to be acceptable based on standard methods of evaluation.

Concentrations of ACTH in the spotted dolphins (457.7 ± 307.1 pg/mL, range – 90.1-1532 pg/mL) were significantly higher than in samples voluntarily obtained from captive beluga whales (6.6 ± 5.8 pg/mL, range <0.5 – 24.3 pg/mL) (Schmitt, St. Aubin and Dunn, unpublished) or bottlenose dolphins captured in Sarasota (246.2 ± 219.3 pg/mL, range 47.4 – 870 pg/mL). The dynamics of this hormone in odontocetes have not yet been determined. However, elevations are detected in humans within 10-30 minutes after

stimulation by exogenous corticotropin releasing hormone or intense exercise, and then diminish as cortisol levels rise (Orth and Kovacs 1998, Singh *et al*. 1999, Deuster *et al*. 2000). The apparent persistence of significantly elevated levels of ACTH in spotted dolphins implies that confinement within the net, or activities associated with swimmers, handling and sampling for this study, constituted an on-going source of acute stress and stimulation of the hypothalamic-pituitary axis for these animals.

Stimulation of the adrenal medulla is typically one of the first events in the mammalian stress response. The release of catecholamines, principally epinephrine (E), has a host of cardiovascular, visceral and metabolic effects that contribute to a general state of preparedness to flee or fight (Young and Landsberg 1998). Other catecholamines, such as norepinephrine (NE) and dopamine (DA), are primarily neurotransmitters, and their appearance in circulation typically reflects enhanced neurological activity. As for some of the other constituents analyzed in this study, published data for catecholamine levels in odontocetes are meager. Thomas *et al*. (1990) reported levels of 0-101 pg/mL for E and 160-604 pg/mL for NE in captive belugas, both resting and subjected to high amplitude playbacks of drilling rig noise. By contrast, free-ranging beluga whales captured after 5-15 minute chase had levels averaging 634 and 1423 pg/mL for E and NE, respectively (St. Aubin and Geraci, unpublished). Levels for the spotted dolphins showed particularly large variation, with mean levels of E and NE lower than for the wild-caught belugas, but maximum values higher than for the same wild belugas. Bottlenose dolphins captured in Sarasota had significantly lower levels of both NE and DA than in the spotted dolphins; there was no difference in concentrations of E (Figs. 7 and 8). The findings in spotted dolphins are therefore indicative of an ongoing adrenergic stress response and consistent with the consequences of exertion during the chase.

Total white blood cell counts for the baseline population were higher on average than values reported for a variety of captive odontocetes but typical of samples drawn from free-ranging animals (Bossart *et al*. 2001, St. Aubin *et al*. 2001). Captive odontocetes generally have lower white blood cell counts, usually because captive specimens have been treated for parasitic infections that are responsible for the elevated eosinophil counts often found in wild animals (Cornell *et al*. 1988, Asper *et al*. 1990). In some of the spotted dolphins, total WBC counts exceeded 15,000 cells / mm^3, levels that are associated only with active infection in captive odontocetes. We could not ascertain the nature of the presumed illness.

Blood cellular changes characteristic of the stress response are termed the "stress leucogram", which consists of depressions of eosinophil and lymphocyte counts (eosinopenia and lymphopenia) that are offset by increased numbers of circulating neutrophils (neutrophilia) and result in a net elevation in total white blood cell count (leucocytosis). These changes have been documented in other species of odontocetes following the stress of capture or after exogenous stimulation with ACTH (Thomson and Geraci 1986, St. Aubin and Geraci 1989, 1990). Detection of such shifts within the baseline values for spotted dolphins is complicated by large individual variation in the counts of the various cell types. The relative proportion of cell types does not suggest marked overall differences from average values for odontocetes, although there clearly were some individuals with cell distributions suggestive of stress leucograms.

Other constituents for which nominal baseline values are suspect include the suite of enzymes that are found in muscle tissue and released into circulation following excessive

exertion with lysis of muscle fibers. Mean levels of CK, AST, and LDH were all elevated relative to reported values for several species of odontocetes (Bossart *et al*. 2001) but similar to those reported for net-impounded harbor porpoises (Koopman *et al*. 1995). These changes raise the possibility of "capture myopathy", which in some species has fatal consequences (Spraker 1993). Some individuals were more noticeably affected with particularly high values for one or other of these indicators, but there was no consistent pattern of concordance among the three enzymes. In general, it is difficult to directly correlate serum enzyme levels with impaired muscle function (Margaritis *et al*. 1999), however levels associated with massive necrosis are considerably higher (Sayers *et al*. 1999) than those observed in the dolphins. None of these indices was correlated with measures of acid-base imbalance (i.e., bicarbonate, anion gap, electrolytes) to suggest a suite of changes associated with metabolic acidosis. In other species of dolphins, such changes in muscle enzymes are considered subclinical forms of myopathy, which typically resolves in a few days (G. Bossart, pers. comm.).

The temporal heterogeneity in the baseline data afforded an opportunity to determine whether any of the perturbations presumed to be indicative of a stress response were developing within the 2.5 hour sampling window, which began more than 1.5 hours after the start of the chase. For some of the constituents examined, such as cortisol, glucose, and the various leucocytes, this time interval falls squarely within the period of maximal effect. It might be expected that animals subjected to the continuous stress of confinement and gradual constriction of the space around them would express these changes to an increasing degree over time. In fact, few such associations were detected statistically, and several of those that were ran counter to the anticipated direction of change. For example, lymphocyte and eosinophil counts increased rather than decreased in the later samples, as did levels of T3 and fT4. Only glucose showed the expected rise with time after the onset of the stress response. The possibility remains that individual dolphins were experiencing the classic set of hormonal and physiological responses, but that an analysis across the group based on single samples from each individual was unable to detect such changes due to wide individual variation in the initial values for the constituents.

Other important observations emerge from the analysis of changes over time. Of particular interest are the pituitary-adrenal axis, and catecholamine responses. In the former, the appearance of ACTH in the bloodstream elicits a response from the adrenal cortex, resulting in the secretion of cortisol and, in marine mammals particularly, aldosterone (Thomson and Geraci 1986, St. Aubin and Geraci 1990, St. Aubin 2001, St. Aubin and Dierauf 2001). A feedback mechanism exists wherein elevations of cortisol exert a negative effect on the release of ACTH, thereby maintaining a physiologically appropriate balance between the two hormones. We might therefore expect that elevations in the levels of ACTH would occur in the earliest samples while cortisol levels were still relatively normal. Later, high levels of cortisol might be associated with reduced concentrations of ACTH, signifying that the individuals were accommodating to the initial stress of the chase and encirclement. In the samples collected from spotted dolphins, neither ACTH nor cortisol levels were correlated with time. This suggests that the endocrine response triggered by the central nervous system was not diminished during the course of encirclement, and that some individuals continued to exhibit an acute stress response hours after the initial stressful stimulus (Fig. 3). By contrast, data collected from bottlenose dolphins sampled in Sarasota demonstrate the expected acute pituitary-adrenal

response, with the highest ACTH concentrations occurring soon after capture, while cortisol levels are low, and lower ACTH later as cortisol levels increase (Fig. 3). We have no specific information on what levels ACTH and cortisol might attain in bottlenose dolphins subjected to the same extended stress of confinement experienced by the spotted dolphins, but the large differences in the levels of both constituents between the two species suggest an exaggerated response by the latter.

The lack of a consistent temporal association in the levels of aldosterone is perplexing, given the consistency of the response of this hormone to stimulation by exogenous ACTH in cetaceans (Thomson and Geraci 1986, St. Aubin and Geraci 1990). It has been hypothesized that the release of aldosterone as part of the stress response in marine mammals is an adaptation to maintain fluid and electrolyte balance under duress (Geraci 1972, St. Aubin and Geraci 1986, St. Aubin *et al*. 1996, St. Aubin and Dierauf 2001). Repeated stimulation of the adrenal cortex by ACTH can lead to a state known as "aldosterone escape", in which the zona glomerulosa shows a diminished response, and this may account for the deviation from the expected pattern. Still, the levels of aldosterone measured in the spotted dolphins are moderately elevated compared with those considered to be basal in other species of cetaceans (St. Aubin 2001), and may reflect the effects of the continued high concentrations of ACTH. Elevations in aldosterone do not likely represent the same negative feedback stimulus to the pituitary as do changes in cortisol.

The persistent elevations of the catecholamines in the spotted dolphins are also noteworthy. These substances are usually characterized by rapid clearance from the bloodstream (Young and Landsberg 1998). The presence of the sampling rafts and activities of the swimmers could well explain almost instantaneous and repeated spikes in E throughout the sampling period. However, levels of norepinephrine had sufficient time to normalize if the chase had been the principal stimulus for release of this neurotransmitter. Dolphins in the seine net are continually swimming, but not exerting themselves to the degree that is documented during the chase (Pabst *et al*., 2002). The precise role of dopamine, whether as part of a neurally- or endocrine based system, has not been resolved, in part because resting levels are typically very low in the blood (Young and Landsberg 1998). The marked and prolonged elevations of this substance in the spotted dolphins is therefore enigmatic at this time.

An important consideration in this analysis is the marked individual variation that was expected in the blood profiles of the dolphins. Indeed, many constituents demonstrated outlying values indicative of a physiological state that was significantly different from that for other dolphins experiencing the same stressful stimulus. Adrenocortical stress responses differ systematically in subsets of the human population (Petrides *et al*. 1997, Singh *et al*. 1999) and changes in circulating hormones and indicators of muscle damage following exercise vary considerably with the training and experience of the individual (Luger *et al*. 1987, Deuster *et al*. 1989, Viru *et al*. 2001). It is possible to identify dolphins that exhibited unexpectedly large deviations in catecholamines, muscle enzymes and electrolyte or acid-base balance, and these generally comprised 5-10% of the individuals sampled. However, the findings themselves do not allow any prediction regarding the ability of these animals to deal with their altered status. To complete the interpretation and develop a prognosis for these individuals requires serial sampling, as proposed in the original protocol for this study. This was the third, and perhaps most important, objective of the blood analyses.

In all, sixteen dolphins were recaptured and sampled on at least one occasion. However, six of these had been fitted with transmitters and data loggers to direct recapture efforts and to support ancillary studies, and could not be considered as representative of dolphins affected only by the tuna fishery. Of the remaining ten animals, eight were not blood-sampled the first time they were caught, but only biopsied and fitted with small identifying plastic tags. Thus, only two individuals allowed even a glimpse of how successive captures might influence indicators of acute and chronic stress. For the rest, comparisons made with reference data for the larger population have limited clinical value without any individual baseline.

On first inspection, the changes in the two resampled dolphins appear contradictory. However, some of the differences can be explained by when the samples were collected, relative to both the time from the start of the chase for each capture and the time between captures. Although the second samples for each dolphin were collected at roughly the same times relative to the start of the chase (217 and 201 minutes for D67, and 135 and 139 minutes for D34 for first and second captures, respectively), the difference of over an hour between the animals likely contributed to some of the discrepancies. Beyond this, and perhaps more importantly, D67 was resampled one day after its initial capture, whereas D34 was recaptured on the second day. The higher levels of cortisol in D67 might suggest either an exaggerated response on second capture or an additive effect if elevations resulting from the first capture had not returned to baseline after one day. Declines in T4 and T3, along with elevations in rT3, which are not expected to occur appreciably during the 2-3 hour interval between chase and sampling, are consistent with cortisol-mediated changes (Bianco *et al*. 1987, St. Aubin and Geraci 1988, 1992) that were still being expressed in D67, but had normalized or rebounded in D34 when it was sampled after two days. The depression in iron, and consequently the lower percent saturation of iron binding proteins and higher value for unbound iron binding capacity (UIBC), that is typically associated with stress and the challenge of microbial infection, are also evident in D67 after one day but appear to have recovered in D34. Lower cholesterol, triglycerides and urea in D67 suggest that the dolphin had not been feeding during the one day interval since its first capture, whereas these analytes were elevated or unchanged in D34, indicating that it had resumed feeding. Capture times for these two animals were all within a 90 minute time window in the late morning, and within 20-30 minutes for each pair of samples from a particular dolphin, reducing the possibility that the differences were due to time of day or post chase interval.

Taken as a group, the ten dolphins that were sampled when captured and handled for the second time showed few statistically significant differences from the values determined from first capture samples. Only T4, which declined in the one dolphin (D67) resampled after one day, showed the same trend when all six dolphins recaptured after one day were compared with the baseline population. The limitations in using this strategy for statistical analysis were anticipated, given the wide individual variation that was expected within the general population and the relatively small number of recaptured dolphins. Diagnostically important changes could well be hidden within these data, but cannot be extracted in the absence of first-capture baseline data for each individual. Still, none of the hematological or serum chemical constituents measured in recaptured dolphins showed levels that, by themselves, suggested overt physiological or endocrinological dysfunction.

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LITERATURE CITED

Asper, E.D., L.H. Cornell, D.A., Duffield, D.K. Odell, B.E. Joseph, B.I. Stark and C.A Perry. 1990. Hematology and serum chemistry values in bottlenose dolphins. Pages 479-485 *in* S. Leatherwood and R.R. Reeves, eds. The bottlenose dolphin. Academic Press, San Diego, CA.

Beltran, J.F., M. Recio and C. Aza. 1991. Hematological and serum chemical characteristics of the Iberian lynx (*Lynx pardina*) in south-western Spain. Canadian Journal of Zoology 69:840-846.

Bianco, A.C., M.T. Nunes, N.S. Hell and R.M.B. Maciel. 1987. The role of glucocorticoids in the stress-induced reduction of extrathyroidal 3,5,3'-triiodothyronine generation in rats. Endocrinology 120:1033-1038.

Borjesson, D.L., M.M. Christopher and W.M. Boyce. 2000. Biochemical and hematologic reference intervals for free-ranging desert bighorn sheep. Journal of Wildlife Diseases 36:394-300.

Bossart, G.D., T.H. Reidarson, L.A. Dierauf and D.A. Duffield. 2001. Clinical pathology. Pages 383-436 *in* D.A. Duffield and F.M.D. Gulland, eds. Marine Mammal Medicine Second edition. CRC Press, Boca Raton, FL.

Cornell, L.H. 1983. Hematology and clinical chemistry values in the killer whale (*Orcinus orca)*. Journal of Wildlife Diseases 19:259-264.

Cornell, L.H., D.A. Duffield, B.E. Joseph and B. Stark. 1988. Hematology and serum chemistry values in the beluga (*Delphinapterus leucas*). Journal of Wildlife Diseases 24:220-224.

Curry, B.E. 1999. Stress in mammals: the potential influence of fishery-induced stress on dolphins in the eastern tropical Pacific Ocean. NOAA Technical Memorandum NMFS, NOAA-TM-NMFS-SWFSC-260. Southwest Fisheries Science Center, La Jolla, CA, 121 pp.

Deuster, P.A., G.P. Chrousos, A. Luger, J.E. DeBolt, L.L. Bernier, U.H. Trostmann, S.B. Kyle, L.C. Montgomery and D.L. Loriaux. 1989. Hormonal and metabolic responses of untrained, moderately trained, and highly trained men to three exercise intensities. Metabolism 38: 141-148.

Deuster, P.A., J.S. Petrides, A. Singh, G.P. Chrousos and M. Poth. 2000. Endocrine response to high-intensity exercise: dose-dependent effects of dexamethasone. Journal of Clinical Endocrinology and Metabolism. 85: 1066-1073.

Dunbar, M.R., P. Nol and S.B. Linda. 1997. Hematologic and serum biochemical reference intervals for Florida panthers. Journal of Wildlife Diseases 33:783-789.

Geraci, J.R. 1972. Hyponatremia and the need for dietary salt supplementation in captive pinnipeds. Journal of the American Veterinary Medical Association 161: 618-623.

Kawauchi, H.; K. Muramoto and J. Ramachandran. 1978. Isolation and primary structure of adrenocorticotropin from several species of whales. International Journal of Peptide and Protein Research. 12: 318-324.

Koopman, H.N., A.J. Westgate, A.J. Read and D.E Gaskin. 1995. Blood chemistry of wild harbor porpoises (*Phocoena phocoena)* (L.). Marine Mammal Science 11:123-135.

Koopman, H.N., A.J. Westgate and A.J. Read. 1999. Hematology values of wild harbor porpoises *Phocoena phocoena*(L.). Marine Mammal Science 15: 52-64.

Luger, A., P.A. Deuster, S.B. Kyle, W.T. Gallucci, L.C. Montgomery, P.W. Gold, D.L. Loriaux and G.P. Chrousos. 1987. Acute hypothalamic-pituitary-adrenal responses to the stress of treadmill exercise. Physiologic adaptations to physical training. New England Journal of Medicine 316: 1309-1315.

MacNeill, A.C. 1975. Blood values for some captive cetaceans. Canadian Veterinary Journal 16:187-193.

Margaritis, I., F. Tessier, F. Verdera, S. Bermon and P. Marconnet. 1999. Muscle enzyme release does not predict muscle function impairment after triathlon. Journal of Sports Medicine and Physical Fitness 39: 133-139.

Orlov, M.V., A.M. Mukhlya and N.A. Kulikov. 1988. Hormonal indices in the bottle-nosed dolphin (*Tursiops truncatus)* in the norm and in the dynamics of experimental stress. Soviet Journal of Evolution Biochemistry and Physiology 24:431-436.

Orth, D.N. and W.J. Kovacs. 1998. The Adrenal Cortex. Pages 517-664 *in* J.D. Wilson, D.W. Foster, H.M. Kronenberg, P.R. Larsen eds. Williams Textbook of Endocrinology. Ninth edition. W.B. Saunders Co., Philadelphia, PA.

Ortiz, R.M., and G.A.J. Worthy. 2000. Effects of capture on plasma adrenal steroids and vasopressin levels in free-ranging bottlenose dolphins *(Tursiops truncatu*s), Comparative Biochemistry and Physiology. A 125:317-324.

Pabst, D. A., W. A. McLellan, E. M. Meagher, A. J. Westgate. 2002. Measuring temperatures and heat flux from dolphins in the eastern tropical Pacific: Is thermal stress associated with chase and capture in the ETP-tuna purse seine fishery? Administrative Report LJ-02-34C, NMFS, Southwest Fisheries Science Center, 8604 La Jolla Shores Drive, La Jolla, CA 92037.

Petrides, J.S., Gold, P.W., Mueller, G.P., Singh, A., Stratakis, S., Chrousos, G.P., and P.A. Deuster. 1997. Marked differences in functioning of the hypothalamic-pituitary-adrenal axis between groups of men. Journal of Applied Physiology 82:1979-1988.

Ridgway, S.H., J.G. Simpson, G.S. Patton and W.G. Gilmartin. 1970. Hematologic findings in certain small cetaceans. Journal of the American Veterinary Medical Association. 157:566-575.

St. Aubin, D.J. 2001. Endocrinology. Pages 165-192 *in* L. A. Dierauf and F.M.D. Gulland eds. Handbook of Marine Mammal Medicine: Health, Disease and Rehabilitation. Vol. 2 CRC Press, Boca Raton, FL.

St. Aubin, D.J. and L.A. Dierauf. 2001. Stress in Marine Mammals. Pages 253-269 *in* L. A. Dierauf and F.M.D. Gulland eds. Handbook of Marine Mammal Medicine: Health, Disease and Rehabilitation. Vol. 2. CRC Press, Boca Raton, FL.

St. Aubin, D.J., and J.R. Geraci. 1986. Adrenocortical function in pinniped hyponatremia. Marine Mammal Science 2:243-250.

St. Aubin, D.J., and J.R. Geraci. 1988. Capture and handling stress suppresses circulating levels of thyroxine and triiodothyronine in beluga whales (*Delphinapterus leucas)*. Physiological Zoology 61:170-175.

St. Aubin, D.J., and J.R. Geraci. 1989. Adaptive changes in hematologic and plasma chemical constituents in captive beluga whales (*Delphinapterus leucas)*. Canadian Journal of Fisheries and Aquatic Sciences 46:796-803.

St. Aubin, D.J., and J.R. Geraci. 1990. Adrenal responsiveness to stimulation by adrenocorticotropic hormone (ACTH) in captive beluga whales (*Delphinapterus leucas)*. Pages 149-157 *in* T.G. Smith, D.J. St. Aubin and J.R. Geraci, eds. Advances in

Research on the beluga whale (*Delphinapterus leucas)*. Canadian Bulletin of Fisheries and Aquatic Science, vol. 224.

St. Aubin, D.J., and J.R. Geraci. 1992. Thyroid hormone balance in beluga whales (*Delphinapterus leucas)*: dynamics after capture and influence of thyrotropin. Canadian Journal of Veterinary Research 56:1-5.

St. Aubin, D.J., S.H. Ridgway, R.S. Wells and H. Rhinehart. 1996. Dolphin thyroid and adrenal hormones: circulating levels in wild and semidomesticated *Tursiops truncatus*, and influence of sex, age, and season. Marine Mammal Science 12:1-13.

St. Aubin, D.J., S. DeGuise, P.R. Richard, T.G. Smith and J.R. Geraci. 2001. Hematology and plasma chemistry as indicators of health and ecological status in beluga whales (*Delphinapterus leucas)*. Arctic 54:317-331.

Sayers, S.P., P.M. Clarkson, P.A. Rouzier and G. Karmen. 1999. Adverse events associated with eccentric exercise protocols: six case studies. Medicine and Science in Sports and Exercise 31: 1697-1702.

Singh, A., J.S. Petrides, P.W. Gold, G.P. Chrousos and P.A. Deuster. 1999. Differential Hypothalamic-pituitary-adrenal axis reactivity to psychological and physical stress. Journal of Clinical Endocrinology and Metabolism 84: 1944-1988.

Spraker, T.R. 1993. Stress and capture myopaty in artiodactyls. In Fowler, M.E. (ed.) Zoo and Wild Animal Medicine. W.B. Saunders, Philadelphia PA. p. 481-488.

Thomas, J.A., R.A. Kastelein and F.T. Awbrey. 1990. Behavior and blood catecholamines of captive beluga whales during playbacks of noise from an oil drilling platform Zoo Biology 9: 393-402.

Thomson, C.A., and J.R. Geraci. 1986. Cortisol, aldosterone, and leucocytes in the stress response of bottlenose dolphins (*Tursiops truncatus)*. Canadian Journal of Fisheries and Aquatic Sciences 43:1010-1016.

Viru, A.M., A.C. Hackney, E. Valja, K. Karelson, T. Janson and M. Viru. 2001. Influence of prolonged continuous exercise on hormone responses to subsequent exercise in humans. European Journal of Applied Physiology 85: 578-585.

Vogel, I., J.-C. Vié, B. De Thoisy and B. Moreau. 1999. Hematological and serum chemistry profiles of free-ranging southern two-toed sloths in French Guiana. Journal of Wildlife Diseases 35:531-535.

Young, J.B., and L. Landsberg. 1998. Catecholamines and the adrenal medulla. Pages 665-728 *in* J.D. Wilson, D.W. Foster, H.M. Kronenberg, P.R. Larsen eds. Williams Textbook of Endocrinology. Ninth edition. W.B. Saunders Co., Philadelphia, PA.

Table 1. Hematological data from 50 spotted dolphins sampled on first handling during CHESS.

	Units	Mean	SD	95% CI	Median	Range
WBC	10^3 cells/mm^3	10.0	2.5	9.3 -10.7	9.7	5.95 -18.6
Neutrophils	cells/mm^3	5146	1455	4733 -5560	5038	2448 -8265
Bands	cells/mm^3	2	14	-2 -6	0	0 -97
Lymphocytes	cells/mm^3	2460	1318	2086 -2835	2116	213 -7440
Monocytes	cells/mm^3	312	168	265 -360	290.5	0 -836
Eosinophils	cells/mm^3	2021	951	1751 -2291	1857	424 -5580
Basophils	cells/mm^3	79	97	51 -106	64.5	0 -506
Neutrophils	%	52.0	11.7	48.7 -55.3	51.5	29 -79
Bands	%	0.02	0.1	0.0 -0.1	0	0 -1
Lymphocytes	%	24.1	9.7	21.3 -26.9	21.5	3 -42
Monocytes	%	3.2	1.6	2.7 -3.6	3	0 -8
Eosinophils	%	19.9	6.4	18.1 -21.7	19	4 -38
Basophils	%	0.8	0.9	0.5 -1.0	1	0 -4
Platelets	cells/mm^3	150	41.9	138 -162	142.5	17 -253.5
MPV	fm^3	11.1	0.9	10.9 -11.4	11.275	9.4 -13.3
RBC	10^6 cells/mm^3	4.64	0.27	4.56 -4.72	4.6775	4.03 -5.325
HGB	g/dL	16.6	0.8	16.4 -16.9	16.7	15.25 -18.7
HCT	%	46.8	2.4	46.1 -47.4	47.125	42.1 -51.9
Spun HCT	%	46.0	2.6	45.3 -46.8	45.5	41.5 -52.5
MCH	pg	35.9	1.8	35.4 -36.4	35.975	32.4 -41.3
MCHC	g/dL	35.6	0.6	35.4 -35.8	35.6	34.5 -37.4
MCV	fm^3	100.8	4.1	99.7 -102.0	101	92 -110.5
RBC Distribution	%	15.9	0.7	15.7 -16.2	15.9	14.65 -18.3

Table 2. Serum chemical constituents in spotted dolphins sampled on first handling during CHESS and from NMFS studies in 1977-78.

		CHESS DATA (n=52)						1977-78 DATA				
Constituent	Units	Mean	SD	95% CI	Median	Range	Sig.	N	Mean	SD	Med	Range
Na	mEq/L	154.7	2.9	153.9 -155.5	154	150 -171	ns	41	154.9	4.6	156	136 -160
K	mEq/L	4.0	0.7	3.8 -4.2	4	2.8 -6.7	<0.01	44	3.5	1.2	3.15	2 -6.6
Cl	mEq/L	119.3	3.1	118.4 -120.1	120	113 -128	<0.001	44	116.5	3.4	116	109 -126
Na:K		39.4	6.1	37.7 -41.1	39	23 -55	<0.001	41	48.5	13.6	51.667	22.4 -78
Anion Gap	mEq/L	17.3	4.2	16.1 -18.5	17	13 -43		nd				
Bicarbonate	mEq/L	22.2	3.5	21.3 -23.2	22	10 -29		nd				
Ca	mg/dL	8.8	0.5	8.7 -9.0	8.8	7.7 -10	<0.001	44	8.2	0.6	8.25	6.1 -10.1
P	mg/dL	5.7	1.5	5.3 -6.1	5.6	3.1 -9	<0.001	44	3.7	1.2	3.6	1.3 -7.2
Fe	ug/dL	124.4	38.3	113.7 -135.1	124	48 -227	ns	44	114.7	33.0	107	63 -208
TIBC	ug/dL	313.0	52.1	298.4 -327.5	304	232 -442		nd				
UIBC	ug/dL	188.6	54.6	173.4 -203.8	192	70 -326		nd				
% Saturation	%	40.1	12.3	36.7 -43.5	39	21 -74		nd				
Mg	mEq/L	1.6	0.2	1.6 -1.7	1.6	1.3 -2.2		nd				
Glucose	mg/dL	136.1	24.0	129.6 -143.0	135	92 -215	ns	44	139.8	31.0	136.5	87 -230
Urea	mg/dL	67.6	12.3	64.2 -71.0	70	44 -91	ns	44	65.7	10.7	64.5	35 -95
Uric Acid	mg/dL	0.7	0.5	0.6 -0.9	0.7	0.1 -1.8	ns	19	0.9	0.4	0.8	0.3 -1.7
Creatinine	mg/dL	0.8	0.2	0.8 -0.9	0.8	0.4 -1.5	<0.001	44	1.0	0.2	1	0.7 -1.7
Direct Bilirubin	mg/dL	0.1	0.02	0.09 -0.1	0.1	0 -0.2		nd				
Ind. Bilirubin	mg/dL	0.1	0.1	0.06 -0.1	0.1	0 -0.3		nd				
Total Bilirubin	mg/dL	0.2	0.1	0.15 -0.2	0.2	0.1 -0.4	<0.001	44	0.1	0.1	0.08	0.01 -0.36
Cholesterol	mg/dL	232.8	52.3	218.2 -247.4	227	129 -338	<0.001	44	182.4	42.2	175.5	117 -312
Triglycerides	mg/dL	200.4	134.0	163.0 -237.7	166	32 -506	<0.001	43	104.0	72.6	84	13 -362
Total Protein	g/dL	7.2	0.7	7.0 -7.4	7	6.0 -9.0	<0.001	44	6.5	0.5	6.6	5.3 -7.9
Albumin	g/dL	3.7	0.2	3.6 -3.8	3.8	3.0 -4.0	ns	44	3.7	0.3	3.7	3.2 -4.6
Globulin	g/dL	3.5	0.6	3.3 -3.7	3.4	2.7 -5.3	<0.001	44	2.8	0.6	2.75	1.43 -4.4
A:G		1.1	0.2	1.0 -1.1	1.03	0.66 -1.43	<0.001	44	1.4	0.4	1.28	0.8 -2.87
Fibrinogen	mg/dL	368	118	334 -401.6	349	222 -936		nd				
AP	U/L	379.5	192.6	325.9 -433.1	363	75 -935	ns	43	347.1	154.7	333	100 -887
ALT	U/L	127.4	40.6	116.0 -138.7	119	61 -258	<0.01	43	101.4	34.9	102	33 -170
AST	U/L	334.8	67.1	316.2 -353.5	334	182 -520	<0.001	43	268.7	48.6	259	150 -399
Amylase	U/L	1.0	0.3	1.0 -1.1	1	1 -3		nd				
CK	U/L	263.0	90.1	237.9 -288.1	258	127 -560	<0.001	44	88.5	60.1	71.5	9 -261
GGT	U/L	30.6	4.1	29.5 -31.8	30	22 -39		nd				
LDH	U/L	618.3	129.4	582.3 -654.3	609	450 -1308	ns	35	616.7	163.3	599	62 -850

* n = 48 for fibrinogen

Table 3. Serum hormone and protein electrophoresis data from spotted dolphins sampled on first handling during CHESS. Data for reproductive hormones are reported only for the appropriate gender.

Constituent	Units	N	Mean	SD	95% CI	Median	Range
ACTH	pg/mL	50	457.7	307.1	370.4 -545.0	355	90.1 -1532
Cortisol	ug/dL	53	5.06	1.25	4.72 -5.41	4.99	2.57 -7.95
Aldosterone	pg/mL	53	134.8	75.0	114.1 -155.5	126.5	5 -305.5
Epinephrine	pg/mL	49	172.8	223.2	108.6 -236.9	124	5 -1592
Norepinephrine	pg/mL	49	927.5	661.9	737.4 -1117.7	742	270 -4322
Dopamine	pg/mL	49	151.3	86.5	126.4 -176.1	138.5	46 -461
Total T4	ug/dL	50	6.61	2.01	6.04 -7.19	6.13	3.23 -10.87
Total T3	ng/mL	50	1.05	0.30	0.97 -1.13	1.01	0.6 -1.99
Free T4	ng/mL	50	3.08	1.05	2.78 -3.38	2.96	1.55 -6.2
Reverse T3	ng/mL	50	1.45	0.47	1.32 -1.59	1.41	0.53 -2.73
rT3 /T3		50	1.45	0.58	1.29 -1.62	1.28	0.65 -2.94
Testosterone	ng/mL	26	5.5	8.9	1.9 -9.1	1.0	0.02 -35.1
Estradiol	pg/mL	23	16.4	4.2	14.6 -18.2	16.9	8.6 -24.2
Progesterone	ng/mL	24	1.6	4.0	-0.1 -3.3	0.4	0.17 -18.1
Albumin	g/dL	20	3.4	0.2	3.3 -3.5	3.4	3 -3.9
Globulin	g/dL	20	3.8	0.7	3.5 -4.2	3.8	2.4 -5.4
alpha-1	g/dL	20	0.63	0.07	0.60 -0.67	0.64	0.5 -0.78
alpha-2	g/dL	20	0.86	0.2	0.76 -0.95	0.86	0.16 -1.13
beta-1	g/dL	20	0.33	0.09	0.29 -0.37	0.31	0.22 -0.22
beta-2	g/dL	20	0.34	0.07	0.31 -0.38	0.35	0.20 -0.45
gamma	g/dL	20	1.67	0.61	1.39 -1.96	1.68	0.92 -3.02

Table 4. Summary of atypical plasma and serum chemical values in spotted dolphins sampled on first capture. Outliers were established using a statistical program.

	Outlying Values		Outer Percentile Values	
Dolphin	Low	High	Lower 10th	Upper 10th
D14	Mg		ALT	
D18	Mg		Na, Na:K	Globulin
D19		Cortisol, ALT, AST, TIBC, UIBC		rT3, CK, DA
D22		Norepinephrine		DA, Na, globulin
D23	Mg		Cl	Bicarbonate, TIBC, globulin
D24		Cl		Na, ACTH
D27		fT4		Cortisol
D29		CK	Na:K, albumin, creatinine, direct bilirubin	K
D32		ACTH, ALT, AST		
D33		glucose	DA, Na:K, bicarbonate	Epinephrine, CK, K, TIBC
D37		AST, K, total and indirect bilirubin	Na, Na:K, UIBC, creatinine, glucose	ALT, bicarbonate
D39		amylase	K, globulin	Na:K, glucose
D40		DA		CK, rT3
D42	bicarbonate	Epinephrine, norepinephrine, Na, anion gap, Mg, glucose, T3	cortisol	albumin
D45		Na:K	K	fT4, rT3
D47	Mg	Epinephrine, norepinephrine, glucose		Cortisol, bicarbonate, albumin, globulin
D48		DA, T3	ALT, anion gap	
D49		CK	Na, bicarbonate	
D59	albumin		Anion gap, TIBC	ACTH
D220		ALT		Cortisol, AST
D221	Mg, direct bilirubin	globulin	CK, bicarbonate	Epinephrine, norepinephrine, anion gap
D223		Norepinephrine		
D224		Epinephrine	globulin	Na, Cl
D227		ACTH	T3	Na, albumin, creatinine
D228		rT3	creatinine	ACTH
D229		Anion gap, direct bilirubin, creatinine		rT3

Table 5. Time (in minutes) between various events and blood collection for chemistry and hormones for the 17 sets in which dolphins were captured and sampled.

	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5
Mean	151.8	120.1	107.5	60.9	21.0
SD	37.2	21.6	17.3	15.4	11.9
Min	102	90	82	41	7
Max	252	186	170	114	72

Table 6. Serum chemical constituents showing significant differences according to gender and relative age in spotted dolphins.

		Female		Male		Differences (p<)		
Constituent	Units	Immature (6)	Mature (19)	Immature (8)	Mature (18)	Gender	Maturity	Interaction
Albumin	g/dL	3.72 ± 0.15	3.62 ± 0.26	3.65 ± 0.19	3.80 ± 0.17	ns	ns	0.05
ALP	U/L	562 ± 206	218 ± 103	473 ± 115	460 ± 163	0.05	0.001	0.001
Calcium	mg/dL	9.0 ± 0.4	8.6 ± 0.5	9.2 ± 0.5	8.9 ± 0.4	ns	0.001	ns
Cholesterol	mg/dL	202 ± 46	251 ± 46	195 ± 39	242 ± 56	ns	0.001	ns
CK	U/L	261 ± 63	221 ± 67	345 ± 88.0	255 ± 69	0.001	0.001	ns
Creatinine	mg/dL	0.72 ± 0.21	0.82 ± 0.12	0.91 ± 0.14	0.88 ± 0.23	0.01	ns	ns
TIBC	ug/dL	336 ± 59	301 ± 41	348 ± 59	304 ± 54	ns	0.01	ns
UIBC	ug/dL	212 ± 84	185 ± 42	224 ± 61	170 ± 48	ns	0.01	ns
T4	ug/dL	7.54 ± 1.16	4.96 ± 1.13	8.84 ± 2.07	7.09 ± 1.54 *	0.001	0.001	ns
fT4	ng/mL	3.54 ± 0.46	2.20 ± 0.50	4.20 ± 1.02	3.38 ± 0.92 *	0.001	0.001	ns
T3	ng/mL	1.26 ± 0.34	0.88 ± 0.13	1.28 ± 0.34	1.07 ± 0.27 *	ns	0.001	ns
rT3	ng/mL	1.43 ± 0.30	1.13 ± 0.36	1.67 ± 0.52	1.71 ± 0.42 *	0.001	ns	ns

* n = 17

Table 7. Hematological constituents showing significant differences according to gender and relative age in spotted dolphins.

		Female		Male		Differences (p<)		
Constituent	Units	Immature (7)	Mature (19)	Immature (8)	Mature (16)	Gender	Maturity	Interaction
WBC	10^3 cells/mm^3	9.45 ± 2.1	9.36 ± 2.13	11.81 ± 1.54	10.16 ± 3.15	0.01	ns	ns
Seg. Neutrophils	cells/mm^3	4287 ± 1400	5378 ± 1503	5774 ± 1473	4934 ± 1317	ns	ns	0.01
Lymphocytes	cells/mm^3	2959 ± 1112	1789 ± 978	3416 ± 1063	2562 ± 1524	0.05	0.001	ns
% Seg. Neut.	%	44.6 ± 7.1	57.6 ± 10.9	49.4 ± 12.9	50.0 ± 11.5	ns	0.05	0.05
% Lymphocytes	%	31.9 ± 10.3	18.8 ± 8.5	28.7 ± 8.1	24.7 ± 8.7	ns	0.001	0.05
MCV	fm^3	100.8 ± 3.6	102.4 ± 4.4	98.1 ± 3.1	100.3 ± 3.7	0.05	0.05	ns
MCH	pg	36.0 ± 1.7	36.6 ± 2.1	34.7 ± 1.2	35.7 ± 1.3	0.01	ns	ns
Platelets	cells/mm^3	164.4 ± 30.5	140.2 ± 49.2	141.4 ± 26	159.4 ± 42.1	ns	ns	0.05

Table 8. Summary of changes in two tagged spotted dolphins resampled after one and two days. Arrows indicate whether constituent concentrations were increased (-), decreased (¯), or unchanged («) relative to values at first capture.

Constituent	D67 (bullet tag; 1day)	D34 (roto tag; 2 days)
Cortisol	-	¯
UIBC	-	¯
Phosphate	¯	-
Iron	¯	-
% saturation of IBC	¯	-
Cholesterol	¯	-
Triglycerides	¯	-
T3	¯	-
Chloride	¯	¯
Glucose	¯	¯
Urea	¯	«
Fibrinogen	¯	«
LDH	¯	«
Aldosterone	¯	«
T4	¯	«
FT4	¯	«
rT3	-	«
Creatinine	-	«
Total and Direct Bilirubin	-	«

Table 9. Serum constituents showing significant differences between baseline data obtained at first capture and values in 10 dolphins recaptured one to three days later. Recaptured dolphins were fitted with roto-tags for identification, and are not represented in the first-capture dataset.

	First Capture				Recapture (1-3 days)		
Constituent	n	Mean ± SD	Range	p	n	Mean ± SD	Range
Iron	50	124.0 ± 38.9	48 - 227	< 0.01	10	81.0 ± 41.0	23 - 135
% Saturation	50	40.0 ± 12.5	21 - 74	< 0.01	10	28.0 ± 15.1	9 - 50
CK	50	264.6 ± 91.3	127 - 560	< 0.01	10	182.0 ± 40.0	137 - 265
Globulin	50	3.53 ± 0.59	2.7 - 5.3	< 0.05	10	3.13 ± 0.33	2.6 - 3.6
A:G	50	1.07 ± 0.18	0.66 - 1.43	< 0.05	10	1.21 ± 0.12	1.06 - 1.46
T4	48	6.69 ± 2.02	3.23 - 10.87	< 0.05	10	5.02 ± 1.40	3.82 - 7.33
T3	48	1.06 ± 0.29	0.6 - 1.99	< 0.05	10	0.86 ± 0.19	0.50 - 1.14
rT3:T3	48	1.46 ± 0.59	0.64 - 2.93	< 0.05	10	2.11 ± 1.36	1.0 - 5.26

Table 10. Serum constituents showing significant differences between baseline data obtained at first capture and values in six dolphins recaptured one day later. Recaptured dolphins were fitted with roto-tags for identification, and are not represented in the first-capture dataset.

	First Capture				Recapture (1 day)		
Constituent	n	Mean ± SD	Range	p	n	Mean ± SD	Range
CK	50	264.6 ± 91.3	127 - 560	< 0.05	6	183.0 ± 29.0	161 - 240
A:G	50	1.07 ± 0.18	0.66 - 1.43	< 0.05	6	1.24 ± 0.14	1.06 - 1.46
T4	48	6.69 ± 2.02	3.23 - 10.87	< 0.01	6	4.08 ± 0.25	3.82 - 4.41

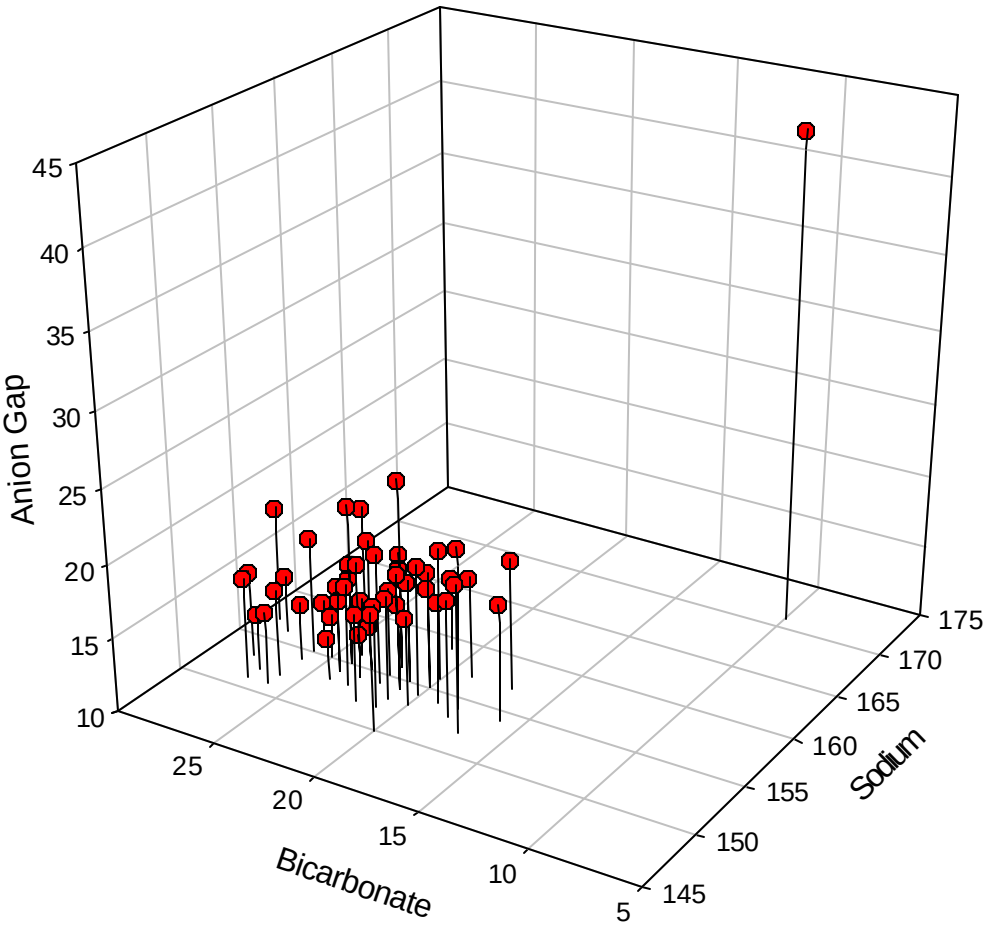


Figure 1a. Distribution of sodium, bicarbonate and anion gap values in spotted dolphins. One individual represented a significant outlier for all three measures.

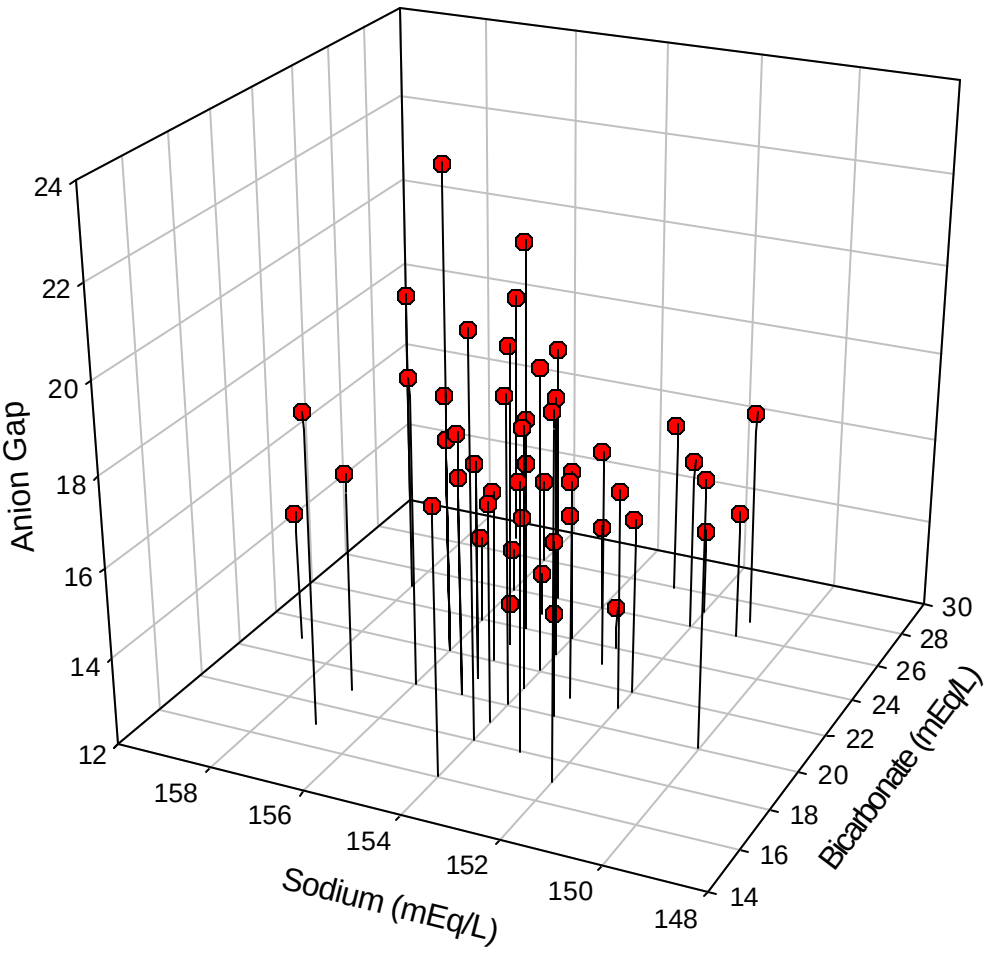


Figure 1b. Distribution of serum sodium, bicarbonate and anion gap in spotted dolphins after removal of one substantial outlier. Bicarbonate was negatively correlated with both sodium and anion gap, which were postitively correlated.

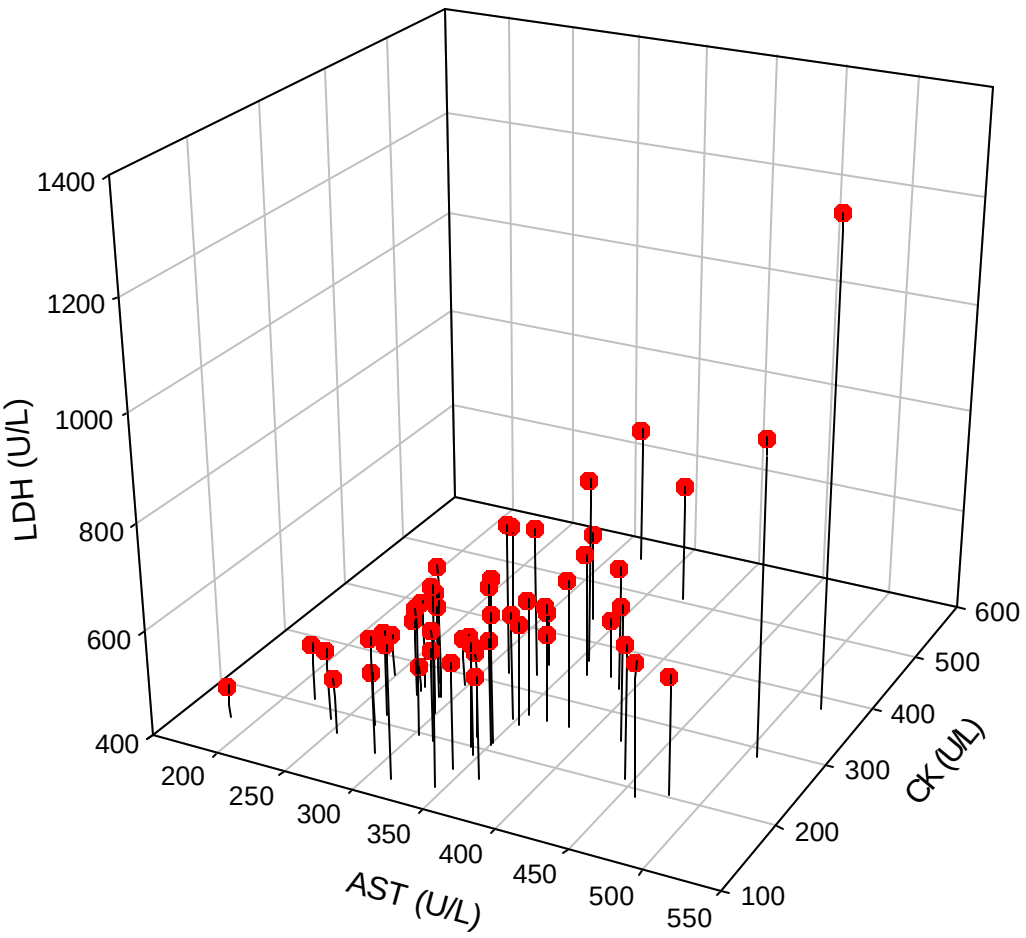


Figure 2. Serum levels of muscle-based enzymes in spotted dolphins sampled after chase and encirclement.

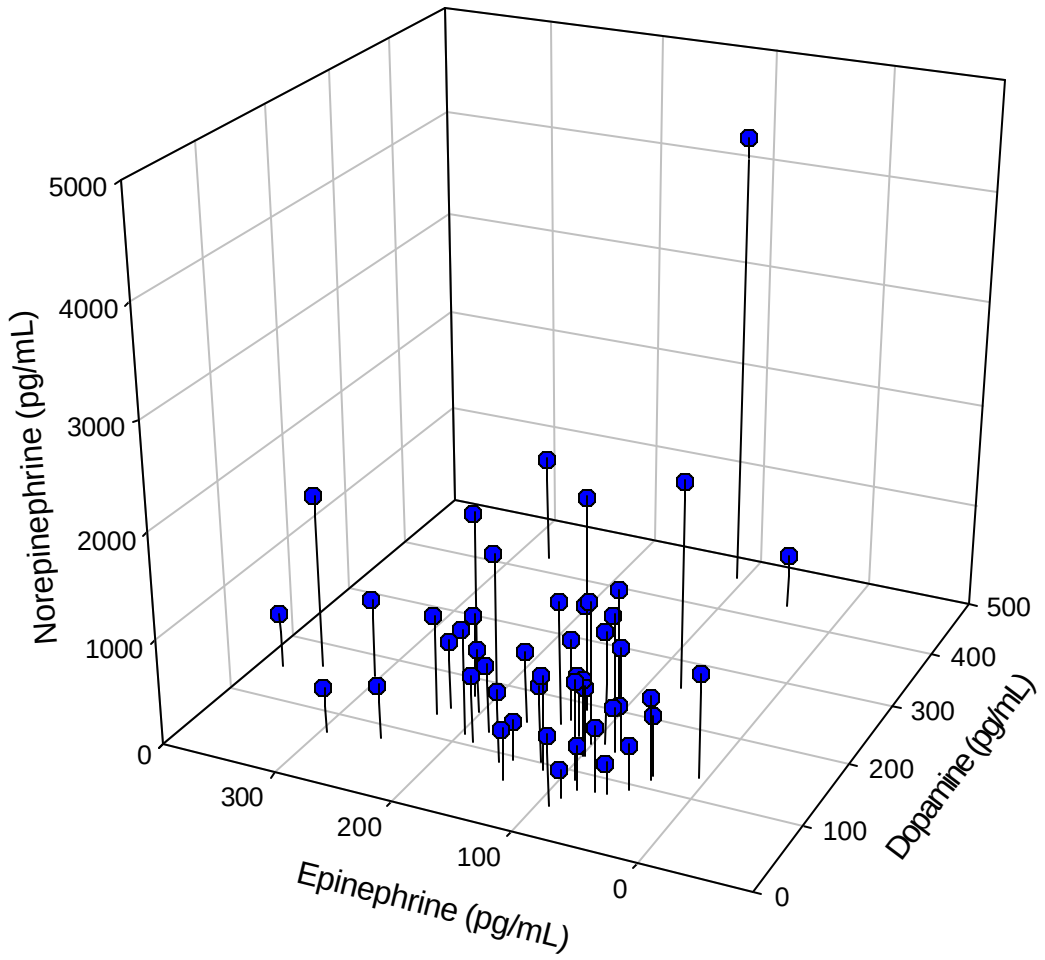
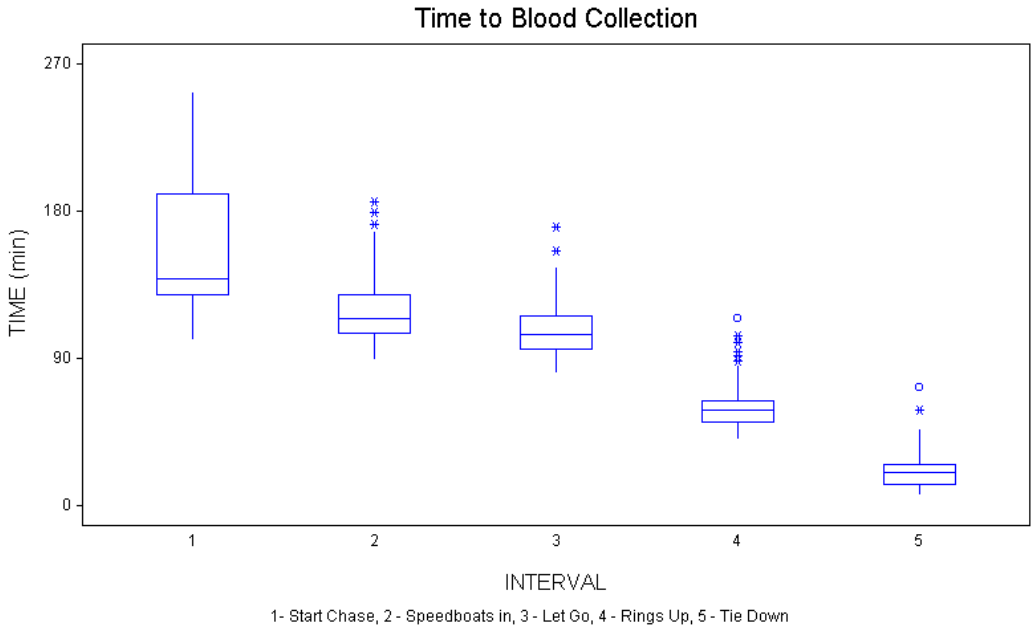


Fig. 3. Plasma catecholamines in spotted dolphins

Figure 4. Box and Whisker plot of time intervals between selected events during the course of the chase and net deployment and the collection of blood samples (see text for details). The boxes enclose half the data, and the horizontal line represents the median value. Whiskers indicate the likely range, with possible outliers shown as asterisks and probable outliers as open circles.



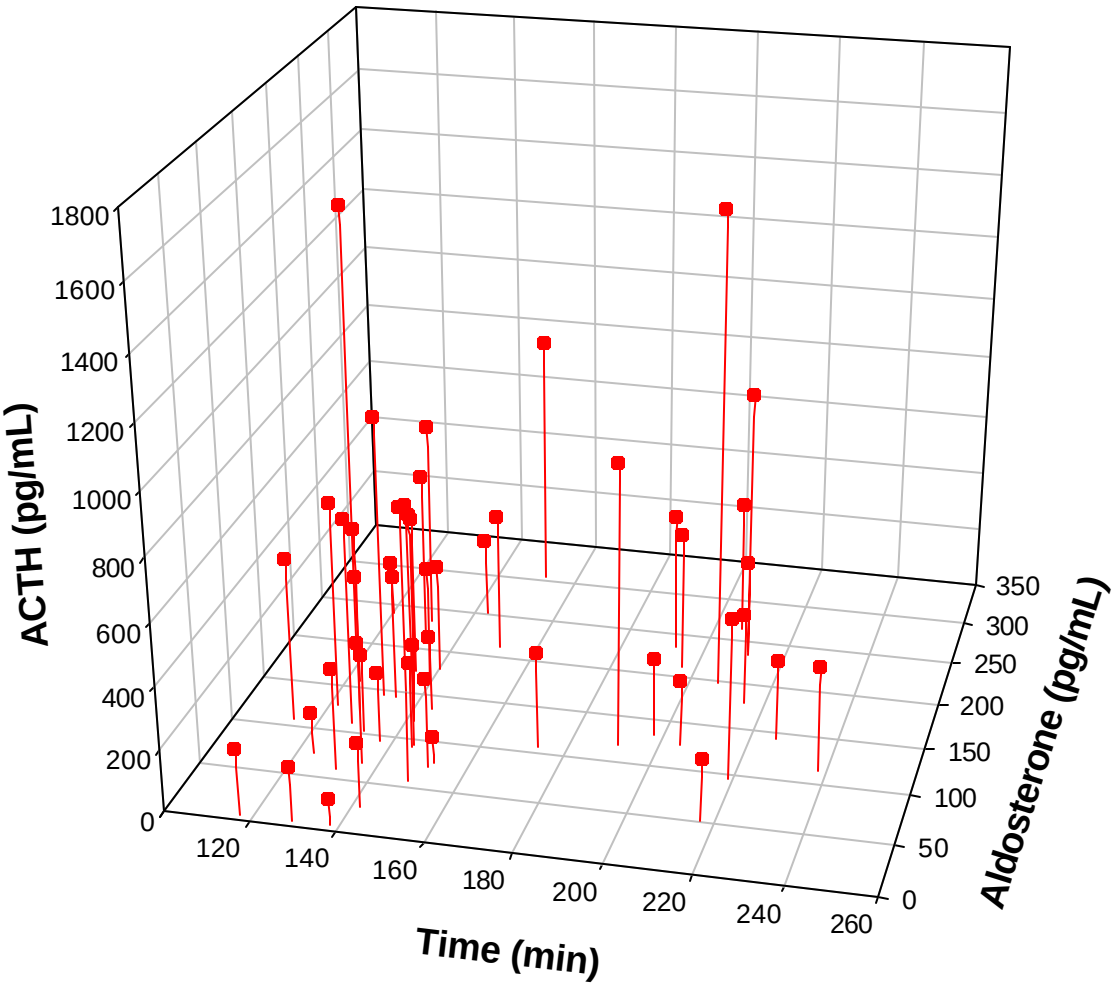


Figure 5. Serum ACTH and aldosterone following chase and encirclement in spotted dolphins.

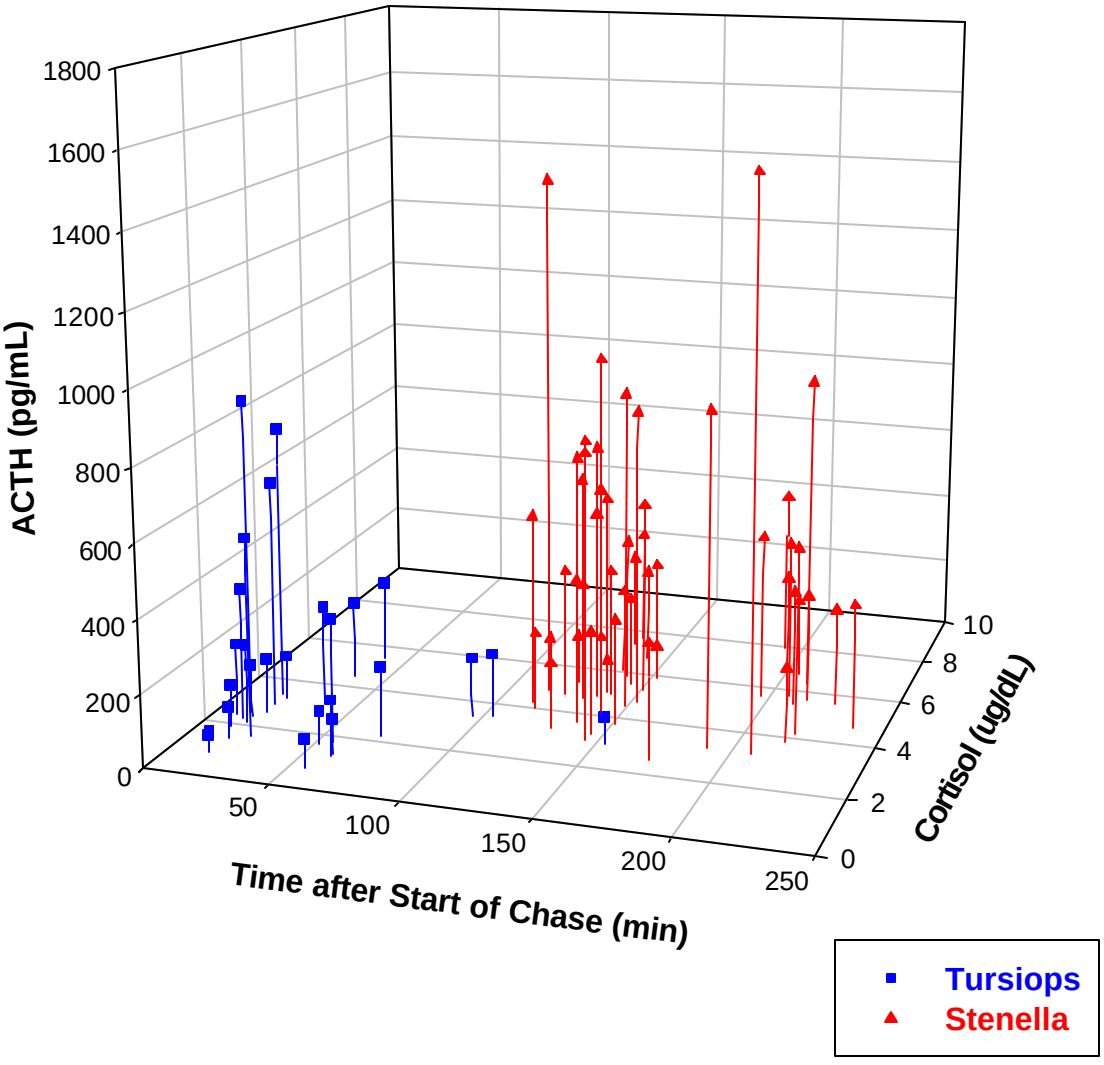


Figure 6. Serum ACTH and cortisol following chase and encirclement in spotted dolphins.

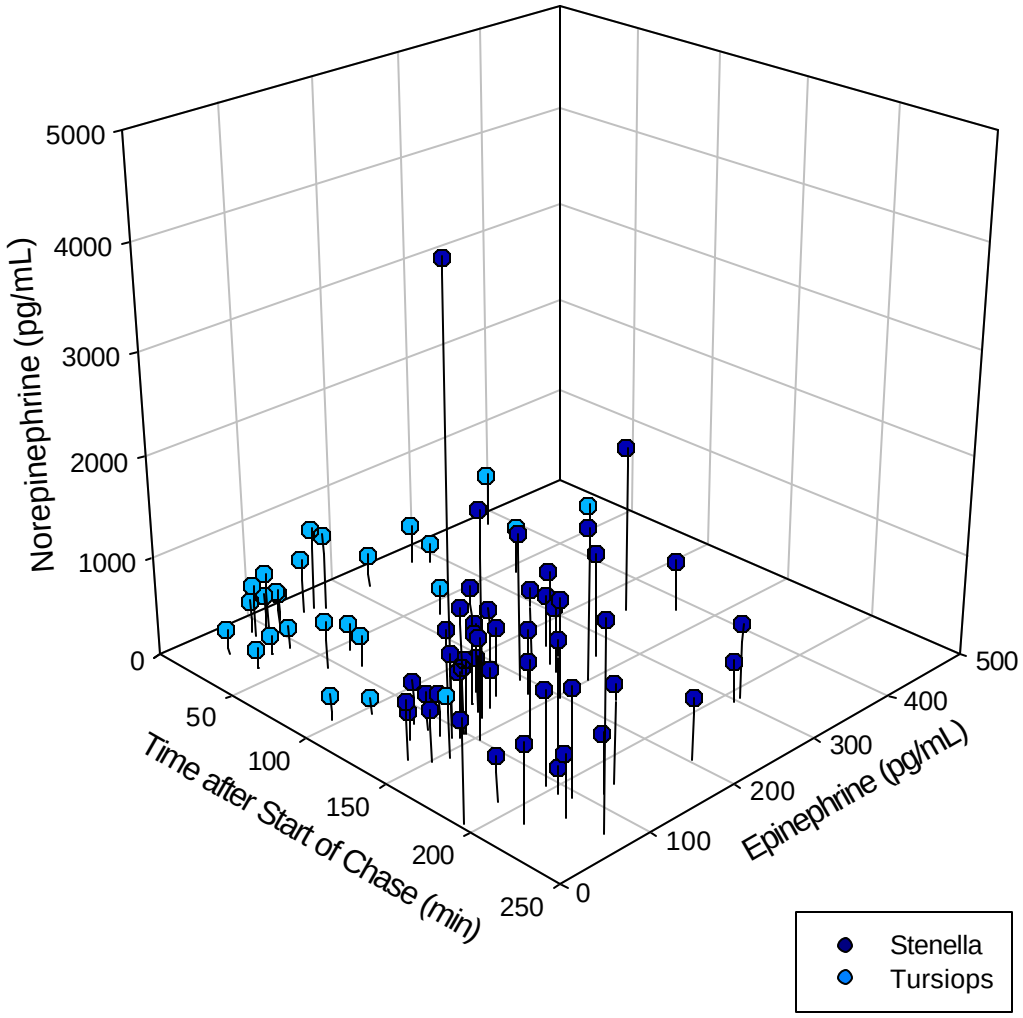


Figure 7. Plasma catecholamines following chase and encirclement in spotted and bottlenose dolphins.

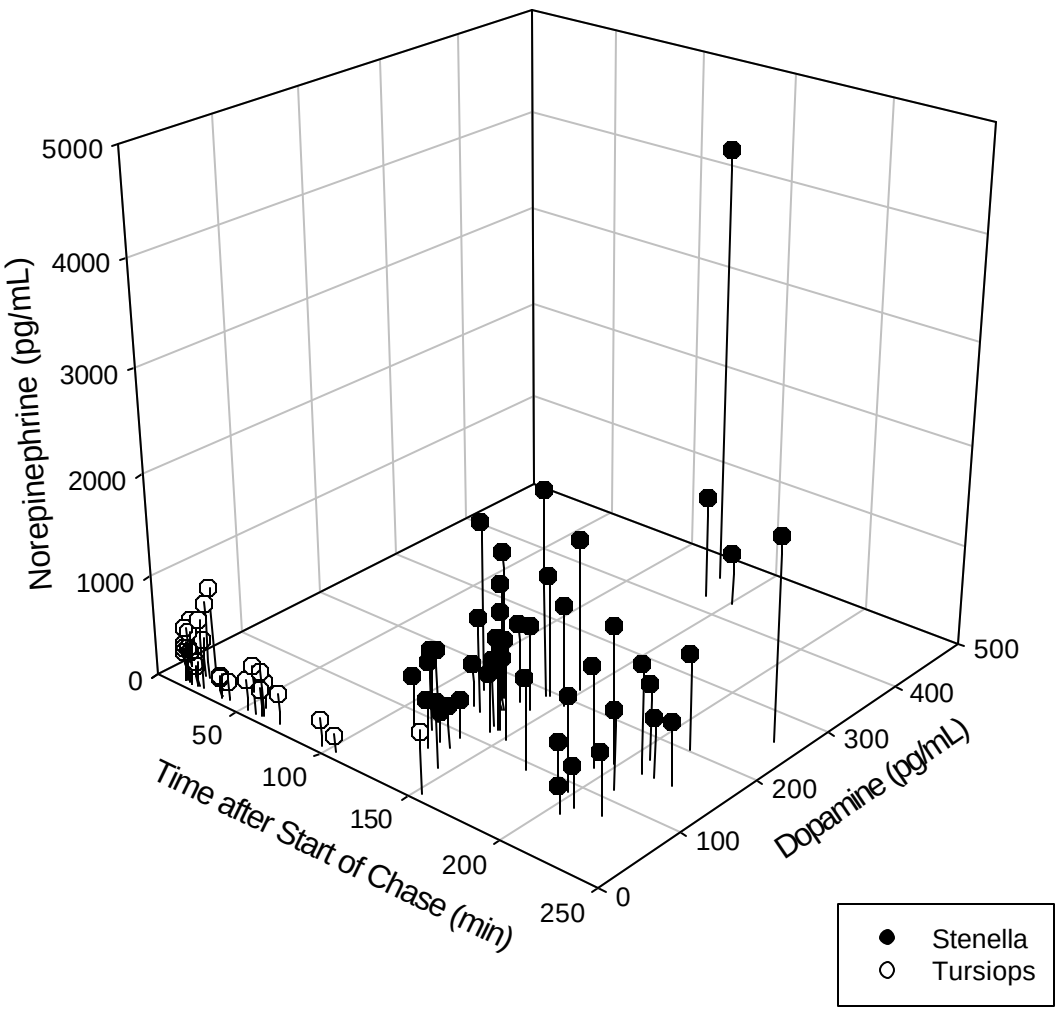


Figure 8. Plasma catecholamines in spotted and bottlenose dolphins following chase and encirclement.

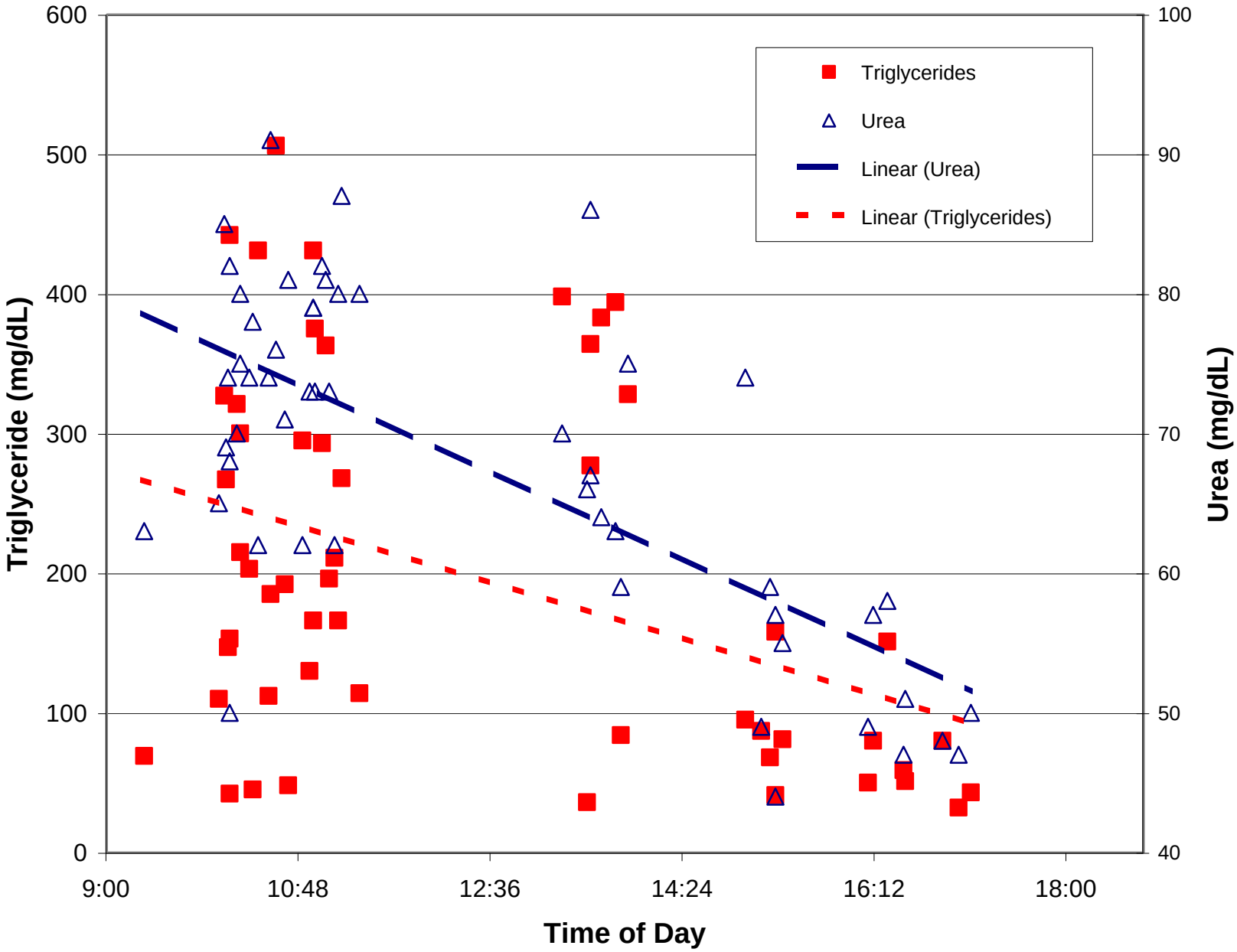


Figure 9. Serum concentrations of triglycerides (solid squares) and urea nitrogen (open triangles) in spotted dolphins sampled at different times of day (local time). Fitted regression lines are both statistically significant.

Appendix 1. Hematological findings in ten dolphins recaptured after one to three days. Two of the animals were also sampled on initial handling. Dolphin 67 was fitted with a bullet tag; all others were roto-tagged.

ID	RBC	HGB	HCT	PLT	MCV	MCH	MCHC	RDW	MPV
1 day									
67 - initial	4.39	15.8	44.3	133	101	36.0	35.7	16.6	11.5
67 - recapture	4.31	15.3	43.4	138	101	35.5	35.2	16.8	11.5
193	4.11	15.5	44.1	89	107	37.7	35.2	16.1	12.6
203	4.64	17.9	51.0	161	110	38.5	35.0	16.8	10.7
209	4.63	16.5	47.3	114	102	35.6	34.8	16.2	12.3
215	4.67	15.3	43.7	117	93	32.7	35.0	16.3	11.8
242	4.28	15.8	44.3	78	104	36.9	35.7	16.4	10.1
2 days									
34 - initial	4.68	17.1	47.4	220	101.5	36.6	36.1	15.4	9.4
34 - recapture	4.69	17.2	47.95	232	102.5	36.7	35.9	15.7	9.6
3 days									
244	4.59	16.7	46.9	125	102	36.3	35.6	15.7	11.6
245	4.58	17.2	48.6	125	106	37.6	35.4	15.9	11.4
257	4.61	16.6	46.6	115	101	36.0	35.6	15.3	11.3

Appendix 1, cont'd.

	Cell Counts							Differential Counts (%)					
ID	WBC	Seg	Band	Eos	Mono	Lymph	Basophil	Seg	Band	Eos	Mono	Lymph	Baso
1 day													
67 - initial	8.5	3910	0	2635	340	1615	0	46	0	31	4	19	0
67 - recapture	8.1	4455	81	1701	405	1458	0	55	1	21	5	18	0
193	7.45	3725	0	1788	224	1714	0	50	0	24	3	23	0
203	7.1	4615	0	710	355	1349	71	65	0	10	5	19	1
209	6.2	3286	0	806	124	1984	0	53	0	13	2	32	0
215	9.65	6659	0	1448	193	1255	97	69	0	15	2	13	1
242	12.5	7125	0	3125	375	1875	0	57	0	25	3	15	0
2 days													
34 - initial	12.9	7611	0	3096	387	1806	0	59	0	24	3	14	0
34 - recapture	12.1	6266	0	2892	241	2410	241	52	0	24	2	20	2
3 days													
244	15.45	11279	0	1700	464	2009	0	73	0	11	3	13	0
245	11	7590	0	1210	330	1760	110	69	0	11	3	16	1
257	9.7	5432	0	2231	388	1552	97	56	0	23	4	16	1

Appendix 2 . Serum and plasma chemical constituents in recaptured spotted dolphins.
Two of the animals (D67 and D34) were also sampled at the time of first capture.

ID	ACTH pg/mL	Aldosterone pg/mL	Cortisol ug/dL	Norepineph pg/mL	Epineph pg/mL	Dopamine pg/mL
1 day						
67 - initial	523	173.62	4.86	758	128	155
67 - recapture	499	21.82	7.74	902	43	106
193	300	169.5	2.51	772	166	140
203	391	136.0	4.84	458	65	97
209	671	148.3	4.56	764	131	152
215	536	172.9	4.33	882	159	160
242	135	155.3	3.14	653	114	99
2 days						
34 - initial	204	50.86	3.86	585	115	151
34 - recapture	44	25.74	2.06	1017	291	310
3 days						
244	955	45.9	4.37	1439	51	111
245	1055	117.9	5.37	1011	131	94
257	1416	170.8	5.49	2032	169	89

ID	T3 ng/mL	rT3 ng/mL	T4 ug/dL	fT4 ng/dL	Testosterone ng/mL	Progesterone ng/mL	Estradiols ng/mL
1 day							
67 - initial	0.91	1.38	5.49	2.96	3.01	-	-
67 - recapture	0.62	2.02	3.82	2.23	1.36	-	-
193	0.93	1.09	3.86	2.08	-	0.23	15
203	1.14	1.29	3.90	2.05	-	0.8	15
209	0.92	1.12	4.21	1.99	1.37	-	-
215	0.76	1.85	4.28	1.86	0.33	-	-
242	1.00	1.00	4.41	1.86	7.45	-	-
2 days							
34 - initial	0.75	0.90	4.24	2.67	-	0.17	14.98
34 - recapture	0.84	0.90	4.79	2.68	-	0.1	10.91
3 days							
244	0.50	2.63	7.33	2.43	13.84	-	-
245	0.97	1.85	7.19	4.14	35.49	-	-
257	0.87	2.34	6.43	3.56	25.65	-	-

Appendix 2, cont'd.

ID	Na mEq/L	K mEq/L	Cl mEq/L	Na:K	Anion Gap mEq/L	Bicarbonate mEq/L
1 day						
67 - initial	154	4.2	124	37	18	16
67 - recapture	155	3.9	117	40	19	23
193	154	4.1	124	38	13	21
203	155	3.7	120	42	15	24
209	154	4.2	121	37	17	20
215	155	4	122	39	18	19
242	153	4.4	122	35	14	21
2 days						
34 - initial	156	4	121	39	17	22
34 - recapture	155	4.1	116	38	19	24
3 days						
244	155	3.8	115	41	16	28
245	153	3.6	110	43	18	29
257	155	4.1	115	38	17	27

ID	Ca mg/dL	P mg/dL	Mg mEq/L	Iron ug/dL	TIBC ug/dL	% Saturation	UIBC ug/dL
1 day							
67 - initial	9	6.7	1.7	155	333	47	178
67 - recapture	9	4.2	1.8	87	331	26	244
193	8.6	5.6	1.7	110	226	49	116
203	8.6	4.2	1.4	134	297	45	163
209	9	6.6	1.9	97	347	28	250
215	8.7	6.6	1.7	80	344	23	264
242	8.8	8.3	1.6	84	370	23	286
2 days							
34 - initial	8.6	5.8	1.7	114	271	42	157
34 - recapture	8.5	6.5	1.8	135	268	50	133
3 days							
244	8.5	4.6	1.5	23	255	9	232
245	8.4	5.2	1.6	35	219	16	184
257	8.9	4.9	1.6	28	245	11	217

Appendix 2, cont'd.

ID	Glucose mg/dL	Urea mg/dL	Uric Acid mg/dL	Creatinine mg/dL	Tot. Bili. mg/dL	Dir. Bili. mg/dL	Ind. Bili mg/dL
1 day							
67 - initial	152	80	1.2	0.7	0.1	0.1	0
67 - recapture	105	50	0.2	1.2	0.5	0.2	0.3
193	135	65	0.8	0.6	0.1	0.1	0
203	136	63	0.5	0.8	0.1	0.1	0
209	122	67	1	0.6	0.1	0.1	0
215	117	68	1.1	0.7	0.1	0.1	0
242	124	71	0.9	0.6	0.1	0.1	0
2 days							
34 - initial	131	80	1.1	0.7	0.1	0.1	0
34 - recapture	115	76	1.4	0.7	0.1	0.1	0
3 days							
244	140	41	<0.2	1.1	0.1	0.1	0
245	173	47	<0.2	1.4	0.2	0.1	0.1
257	213	47	0.2	1.5	0.2	0.1	0.1

ID	Protein g/dL	Albumin g/dL	Globulin g/dL	A:G	Fibrinogen mg/dL	Cholesterol mg/dL	Triglycerides mg/dL
1 day							
67 - initial	7.1	3.9	3.2	1.22	426	263	300
67 - recapture	7.3	4	3.3	1.21	345	186	27
193	6.6	3.7	2.9	1.28	243	263	347
203	6.4	3.8	2.6	1.46	251	296	148
209	7.5	4	3.5	1.14	280	223	251
215	7.1	4	3.1	1.29	312	217	129
242	7.4	3.8	3.6	1.06	175	223	282
2 days							
34 - initial	6.5	3.6	2.9	1.24	391	204	114
34 - recapture	6.3	3.5	2.8	1.25	401	259	158
3 days							
244	6.5	3.6	2.9	1.24	324	127	24
245	6.9	3.7	3.2	1.16	360	221	62
257	7	3.6	3.4	1.06	315	210	93

Appendix 2, cont'd.

ID	ALT U/L	AST U/L	AP U/L	GGT U/L	CK U/L	LDH mg/dL	Amylase U/L
1 day							
67 - initial	77	346	368	33	189	612	1
67 - recapture	75	363	362	32	161	578	1
193	108	326	278	30	184	656	<3
203	122	260	238	32	166	543	<3
209	105	265	792	35	240	619	1
215	154	338	452	32	181	605	1
242	123	309	621	34	167	580	1
2 days							
34 - initial	113	347	344	27	253	605	1
34 - recapture	104	324	349	25	265	541	1
3 days							
244	96	220	130	33	137	406	1
245	99	256	125	36	144	519	1
257	114	299	213	22	172	432	1

APPENDIX 3 – Responses to Reviewer Comments

May 15, 2002

Dr. Karin Forney
Southwest Fisheries Science Center
110 Shaffer Road
Santa Cruz, CA 95060

Dear Karin:

I have carefully reviewed the comments submitted by the panel of expert reviewers on my report entitled "Hematological and serum chemical constituents in pantropical spotted dolphins (*Stenella attenuata*) following chase and encirclement," which was prepared as part of the CHESS program. The reviewers are to be commended for their thorough consideration of the material and helpful suggestions for improvements. Certain points were raised by more than one reviewer, and these will be addressed first before considering specific comments made by each individual.

Lack of proper baseline

From the time the study was conceived, through various planning workshops, and during the experiment itself, it was recognized that interpretation of blood analyses would be confounded by many variables – the stress of chase and encirclement, physical restraint and confinement to sampling rafts, time of day, age, sex, reproductive status, and postprandial status, or even whether a given dolphin had recently been pursued. These limitations were duly noted in the report, and attempts were made in the data analysis to identify the possible contributions of many of these. The study would certainly be improved with the inclusion of a robust dataset for fully-acclimated, captive spotted dolphins, but such data are sparse and not readily available. Furthermore, inconsistencies in methodologies between our study and the few facilities where such animals have been kept would further confound data comparisons. Our expectation in designing this study strategy was that the manipulations involved would represent a relative constant for each recapture, individual animals could serve as their own reference controls, and additive effects of repeated captures might be evident against this background. Ultimately, we were left to make comparisons between spotted dolphins and other species, and between an inadequate number of recaptured animals and a heterogeneous reference dataset. No additional evaluation of the findings will overcome this limitation of the study.

Consideration of Individual Diagnostic Profiles

The reviewers correctly point out that the use of pooled data to assess the health status or stress levels in a population of animals will obscure important correlations among discrete but associated measures in individual animals. This comment is appropriate to our mandate, in that an adverse effect on the larger population might occur even if only a small percentage of individuals within each set experienced some kind of metabolic distress, as evidenced by a recognized pattern of changes in the diagnostic

indicators used. The broad range of analyses performed were intended not only to assess as many metabolic functions as possible, but also to validate diagnoses of organ dysfunction through corroborative determinations.

To this end, data from the first capture samples were reexamined for associations among a variety of indices, particularly those that related to questions raised regarding capture myopathy and metabolic acidosis (see below for more detailed discussion on this point). Some intriguing observations were made on the concurrence of outlying data in certain individuals. In addition, Spearman Rank Correlation tests revealed associations among some of the enzymes, but failed to demonstrate any significant relationship between anion gap or bicarbonate levels (measures of metabolic acidosis) and any of the enzymes considered to be indicative of myopathic changes. These findings bear significantly on the interpretation of the acute effects of chase and encirclement, and the report has been revised to address these observations.

Occurrence and Severity of Capture Myopathy

The reviewers uniformly commented on the interpretation of data suggesting capture myopathy. Opinions differed regarding the severity of the damage and its outcome. The report noted that levels of muscle enzymes were elevated above those reported for other odontocetes, except those that had been subjected to chase and capture. There is no argument with the conclusion that some degree of myopathy occurs in these animals, and this was stated in the report. Reviewers noted that the observed levels "pale in comparison to values observed in clinical rhabdomyolysis" (Dr. Ortiz) and that such "subclinical forms ... usually resolve within a few days" (Dr. Bossart). Dr. Martineau considered the changes to be more serious, and cited numerous examples of debilitating or fatal outcomes of this condition, particularly in ungulates which appear to be more sensitive to this condition than are other mammals. (Dr. Martineau's points about the evolutionary connection between cetaceans and ungulates, and their similar sensitivities to morbilliviruses, should be qualified by the 60 million-year divergence of these groups, changes in fundamental physiological characteristics [cetaceans are not ruminants], and the fact that carnivores are also susceptible to morbilliviruses.)

To a large degree, the issue becomes one of terminology and presumed outcome. The variable manifestations of capture myopathy range from acute capture shock (immediate death) to forms with onset delays of days to a week (Spraker 1993). It is not possible to predict from the current serum chemical evidence whether any of the dolphins sampled might succumb several days later (none of the tracked animals did, and the few resampled dolphins did not show any suggestion of ongoing muscle necrosis). If extensive and potentially fatal muscle necrosis were an outcome in some animals, it might be suggested that at least some of the dolphins necropsied as part of the CHESS program or earlier efforts would show evidence of repaired tissue as visible scarring (one of the results of CM described by Spraker 1993, and described by Dr. Martineau as "white and hard to cut, which reflects scarring characterized by fibrosis"). Some of these animals would be rendered more susceptible to predation, as pointed out by Dr. Martineau, but it is ambitious to suggest that none of them has survived to be observed with gross evidence of scarred muscle (Cowan and Walker 1979, cited by Dr. Martineau).

The opinion that the degree of myopathy experienced by chased and encircled spotted dolphins is "benign" is therefore based on the relatively mild elevations in muscle

enzymes that were observed, and the lack of clinical evidence for sustained muscle necrosis in the few recaptured animals. Still, the possibility that selected individuals may be experiencing more pronounced effects has been recognized in the revised document. Further discussion on this point is provided below.

Specific Comments

Dr. Bossart

As suggested, serum samples have been submitted to the diagnostic laboratory at Cornell University to elucidate the nature of the lower levels of globulins in recaptured dolphins. The findings are included in the revised report.

Dr. Mann

No specific comments to address.

Dr. Martineau

The relationship among the determinants of anion gap was explored in more detail, following Dr. Martineau's suggestion, and revealed a particularly interesting pattern in one outlying individual. These data and a supporting figure have been included in the revised report. No further consideration of the question of metabolic acidosis is possible from the available data and samples. The possibility of determining lactic acid concentrations and blood pH was discussed at planning workshops convened by NMFS, but the expert opinion was that the mixed arterio-venous blood samples that are typically obtained from these animals would yield potentially spurious data. Consequently, these determinations were not made. Relative to this point, however, is the understanding that diving mammals, which may periodically experience anaerobic conditions (although not to the degree suggested by earlier literature), are likely better protected from the damaging effects of acidosis than are terrestrial mammals.

Observations made by Colgrove (1978) of myopathy in a transported dolphin were cited to support the suggestion that odontocetes are susceptible to capture myopathy. In my opinion, the conditions experienced by the animal in question have little bearing on the current issue. Earlier, unsophisticated transportations of dolphins typically held the animals in slings that were unsuspended in water. After many hours, muscle stiffness and pressure necrosis of skin were common outcomes. That fact that any animal placed in such conditions experiences myopathy is not surprising, and does little to elucidate the relative susceptibility of odontocetes to CM.

Changes in laboratory methodology were identified as the most likely explanation for the differences in serum enzyme data between the present study and one conducted in 1977-78 by NMFS. Although Dr. Martineau suggested that different capture methods might also have contributed to thiss pattern, I understand that the approaches were fundamentally the same in the two studies.

In the report, data for circulating levels of muscle enzymes in spotted dolphins were compared with those reported for harbor porpoises captured in seine nets afer 1-3 days of impoundment in herring weirs (Koopman et al. 1995). Dr. Martineau undestood the comparison to suggest that the findings in spotted dolphins mimiced those in porpoises sustaining severe muscle damage while caught in nets over a period of several days. In fact, porpoises in weirs swim freely and feed until they are discovered and

removed. The changes in circulating muscle enzymes in these animals most likely reflects the exertion expended during the 30 minute to 2 hour capture procedure that immediately precedes sampling and release. In this respect, they experience a stress rather similar to that studied in the spotted dolphins. The text of the report was revised to more accurately communicate this fact.

Dr. Martineau's concern that it is "difficult to envision how stress could be measured during the course of a single capture-recapture event" is well-founded. The study plan called for multiple recaptures of individuals at 1-3 day intervals for up to 10 days. Logistic obstacles described elsewhere precluded the achievement of that objective.

Dr. Ortiz

Following Dr. Ortiz's recommendation, the data were examined for correlations between cortisol (F) and testosterone (T), and between T and time of day. Elevations in glucocorticoids can suppress testosterone levels, which are normally secreted episodically throughout the day. Dr. Ortiz suggested that the wide range of T concentrations might be explained, at least in part, by these factors. No significant relationships were observed, but the possibility of detecting such associations in the present dataset was remote. Suppression of T by F occurs following a variable time delay, and thus would not be evident within the "first capture" sample. The study plan called for examination of chronic effects of recapture stress on T, but only one male was recaptured and resampled (at the same time of day; T was lower on the second capture) and any conclusions based on a single sample would be highly speculative. The wide range of T concentrations in that group more simply reflects different degrees of sexual maturity.

Comments from the reviewers prompted an expanded analysis of the findings in "first-time" captured dolphins, as a step towards better understanding the acute effects of chase and encirclement. Still, predictions of the outcomes of these conditions remain speculative in the absence of recapture data. I appreciated the efforts made on the part of the review panel to constructively address the interpretations presented in this and accompanying reports.

Sincerely,

David J. St. Aubin, Ph.D.